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RecurIndex-Guided postoperative radiotherapy with or without Avoidance of Irradiation of regional Nodes in 1-3 node-positive breast cancer (RIGAIN): a study protocol for a multicenter, open-label, randomized controlled prospective phase III trial

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ABSTRACT

Introduction

Postoperative radiotherapy in breast cancer patients with 1-3 lymph node metastases, particularly within the pT1-2N1M0 cohort with a low clinical risk of local-regional recurrence (LRR), has incited a discourse surrounding personalized treatment strategies. The Recurrence Index (RI) model capably differentiates patients based on their level of LRR risk. This research aims to validate whether a more aggressive treatment approach can enhance clinical outcomes in N1 patients who possess a clinically low risk of LRR, yet a high RecurIndex-determined risk of LRR. Specifically, this entails postoperative whole breast irradiation (WBI) combined with regional lymph node irradiation (RNI) following breast-conserving surgery (BCS) or chest wall irradiation (CWI) with RNI after mastectomy.

Methods and Analysis

The RIGAIN study is a multicenter, prospective, randomized, open-label, phase III clinical trial that is being conducted in China. In this study, patients with low clinical LRR risk but high RecurIndex LRR risk are randomly assigned in a 1:1 ratio to the experimental group or the control group. In the experimental group, RNI is performed and the control group omits RNI. Efficacy and safety analyses will be conducted, enrolling a total of 540 patients (270 per group). The primary endpoint is invasive disease-free survival, and secondary endpoints include any first recurrence, local-regional recurrence-free survival, distant metastasis-free survival, recurrence-free survival, overall survival, disease-free survival, breast cancer-specific mortality, and assessment of patient quality of life. The study began in April 2023 and will continue for at least 10 years.

Ethics and dissemination

The study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-097-02). It adheres to the Helsinki Declaration and good clinical practice. Approval from the Chinese Human Genetic Resources Office (Reference Number: 2020SQCJ2358).

Trial registration number NCT04069884

STRENGTHS AND LIMITATIONS OF THIS STUDY

This study represents the first international investigation into the value of a multigene model in guiding precision radiotherapy for N1 patients, particularly when clinical and genetic LRR risk assessments are discordant, specifically in cases of patients who are clinically low LRR risk but genetically high-risk.

The trial is designed as a multicenter, open-label, randomized, controlled phase III study, which will enhance the rigor of the investigation and reduce potential bias.

While the follow-up period for this study is set at 5 years, it may be necessary to extend the follow-up to 10 or 15 years to obtain sufficient data on long-term survival benefits.

INTRODUCTION

Patients with 1-3 axillary lymph node metastases constitute approximately 25 to 30% of early operable breast cancer cases. Radiotherapy plays a pivotal role in the comprehensive treatment of breast cancer[1,2]. However, the benefit of postoperative radiotherapy for N1 breast cancer patients, particularly in terms of survival improvement, remains a topic of substantial debate. Studies conducted in the 1990s such as the Vancouver study, DBCG-82b/82c, and the early meta-analysis by EBCTCG (including the N1 subgroup) consistently demonstrated that postoperative radiotherapy significantly enhances disease-free survival (DFS) and overall survival (OS) for patients[3-7]. Consequently, N1 becomes a relative indication for postoperative radiotherapy. The 2011/2014 EBCTCG meta-analysis further suggested that postoperative radiotherapy could convert a 1.5% reduction in the 10-year any first recurrence rate (AFRR) into a 1% 20-year OS benefit[8,9]. The MA20 and EORTC 22922 studies published in 2015[10,11], which focused on T1-2N1 patients, especially those with high clinical risk of LRR and compared postoperative RNI after BCS or without RNI, found that more aggressive postoperative RNI for T1-2N1 patients could result in better distant metastases-free survival (DMFS), DFS, or breast cancer-specific mortality (BCSM). The Vancouver study's 20-year long-term follow-up results demonstrated the long-term OS benefit of postoperative radiotherapy in the N1 subgroup. These milestone studies further reinforced the value and recommendation of postoperative radiotherapy for N1 breast cancer patients, rendering N1 staging a strong relative indication for postoperative radiotherapy and increasing the number of patients actively accepting postoperative radiotherapy. Nevertheless, not all N1 patients can benefit from postoperative radiotherapy. Some real-world retrospective studies reveal limited LRR and/or survival improvement from postoperative radiotherapy, particularly RNI therapy, among certain N1 patients, especially those with relatively low clinical risk. Consequently, the necessity of radiotherapy for clinically low LRR risk N1 patients remains a topic of significant controversy and uncertainty. Clinical practice often presents varying professional recommendations for postoperative radiotherapy in low-risk N1 patients, resulting in the exclusion of a substantial number of patients solely based on traditional clinical and pathological features. However, this omission of RNI could lead to inadequate treatment, with potential implications for tumor recurrence, metastasis, and patient survival. Conversely, a uniform approach of postoperative radiotherapy for all clinically low LRR risk N1 patients would inevitably result in overtreatment and expose patients to additional risks such as radiation-induced injury and related complications, thereby impacting their quality of life[12-15].

When considering low-risk N1 breast cancer patients, then, the primary objective is to identify the actual high-risk patients concealed within the clinically low-risk population and to strategically administer postoperative radiotherapy. This represents one of the essential development directions of early breast cancer "precision radiotherapy" in the future. Achieving individualized and precise radiotherapy depends on the discovery of molecular genetic prediction models that can accurately predict local-regional recurrence risk in a scientific, reliable, and accessible manner. Currently approved multi-gene detection models abroad include Oncotype DX, MammaPrint, and EndoPredict. Oncotype DX is the most representative and extensively utilized multi-gene prognostic analysis method, primarily employed to guide early luminal low-risk patients to avoid adjuvant chemotherapy. Oncotype DX is currently more frequently used in the radiotherapy field to identify low-risk elderly N0 breast-conserving patients exempt from postoperative radiotherapy. Although the predictive value in the N1 population has initially demonstrated some clinical

significance, contradictions exist between various research findings[16-19]. No prospective high-level evidence for multi-gene models predicting RNI benefits in N1 patients is currently available. The clinical trial Tailor RT (MA 39) conducted by the Canadian Cancer Trials Group primarily investigates whether low-risk recurrence patients can be spared from postoperative radiotherapy or RNI. This is currently the only prospective, randomized, controlled phase III study internationally that utilizes a multi-gene predictive model to guide precise radiotherapy for N1 patients. The future research outcomes will primarily be applied to guide the omission of postoperative radiotherapy in clinically low LRR risk and genetically low-risk N1 patients. However, the significance of postoperative radiotherapy for patients with intersecting risks, especially those with clinically low-risk but genetically high-risk profiles, remains uncertain.

RecurIndex is the only risk prediction model developed based on the Chinese population for early-stage breast cancer. Consisting of 18 core genes and 10 IHC4 reference genes, it is capable of independently predicting the risk of LRR and distant metastasis[20-24]. Internal validation studies in Taiwan and external validation studies conducted in Singapore, Hong Kong, and the Fourth Affiliated Hospital of Hebei Medical University in China have all provided strong evidence of RecurIndex's predictive efficacy and its value in guiding radiotherapy for N1 patients[25,26]. Low-risk and high-risk patients identified by RI-LRR had 5-year LRR rates of 0% and 7%, respectively (P=0.0146). Compared to high-risk RI-LRR patients who did not receive postoperative radiotherapy, those who underwent postoperative radiotherapy demonstrated significantly improved rates of LRR and RFS, with percentages of 88.8% vs 74.1% (P=0.0071) and 79.4% vs 59.5% (P=0.0019), respectively. These results clearly indicate the significant benefits of postoperative radiotherapy in this patient population. To date, RecurIndex has become widely recognized and clinically implemented around the Asia-Pacific region. It has been incorporated into the "Expert Consensus on Multigene Testing for Adjuvant Therapy of Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Early Breast Cancer" in China and recommended in the "Chinese Society of Clinical Oncology (CSCO) Guidelines for the Diagnosis and Treatment of Breast Cancer 2022" for guiding precise postoperative radiotherapy in N1 patients. However, further high-level, randomized, controlled phase III clinical trials are needed to validate its clinical applications and expand its usage in the field. The most promising and crucial area for its application lies in guiding precise radiotherapy for N1 breast cancer patients.

In summary, we have begun conducting a multicenter, prospective, randomized, controlled phase III clinical study of individualized precision radiotherapy for clinically low LRR risk breast cancer patients with N1 guided by RecurIndex. This study aims to evaluate patients' local recurrence and distant metastasis risks, primarily investigating whether active postoperative radiotherapy can further improve clinical efficacy in N1 patients with clinically low risk but high RecurIndex LRR risk. The ultimate goal of this study is to provide high-level clinical evidence and reliable multi-gene recurrence risk prediction models to help achieve individualized precision radiotherapy for N1 breast cancer patients.

MATERIALS AND METHODS

The RIGAIN study is a multicenter, prospective, randomized, open-label, phase III clinical trial that is being conducted in China. The overall research process is illustrated in Figure 1. This study aims

to screen postoperative patients with early-stage breast cancer (pT1-2N1M0) who have completed standard systemic therapy and possess eligible pathological specimens for participation. The inclusion and exclusion criteria are listed in Table 1. RecurIndex testing will be performed using postoperative paraffin-embedded tissue sections from the primary lesion. The study is divided into a randomized controlled trial and an observational study based on clinical risk and RecurIndex LRR risk. Patients with low clinical risk but high RecurIndex LRR risk will be randomly assigned in a 1:1 ratio to either the experimental group (RNI) or the control group (No RNI), while patients with low clinical risk and low RecurIndex LRR risk will be included in the observational study. This article primarily focuses on the randomized controlled trial. The study participants will receive the following treatments. Experimental group: For patients who underwent BCS, RNI will be performed in combination with whole breast irradiation (WBI) + tumor bed boost irradiation. For patients who underwent mastectomy, chest wall irradiation (CWI) will be administered in combination with RNI. Control group: RNI will be omitted. For patients who underwent BCS, only WBI + tumor bed boost irradiation will be administered. For patients who underwent mastectomy, both RNI and CWI will be omitted. A comparative effectiveness analysis will be conducted. The study commenced in April 2023 and is expected to continue for a minimum of 10 years.

Randomization Method

Stratified randomization will be used for the randomized study. For the active postoperative radiotherapy trial involving the clinically low LRR risk but high RecurIndex LRR risk population, participants will be stratified by N1 status, surgical method, and enrolling hospital, and then randomized in a 1:1 ratio into the experimental and control groups.

Randomization stratification factors are as follows:

a) N1 status: N1mic or 1-2 LN macrometastasis (including N1sln), or 3 LN macrometastasis;

b) Surgical method: breast-conserving surgery or mastectomy; and

c) Multicenter enrolling hospital.

A central randomization system was developed by TaiMei Medical Technology Company to facilitate the randomization process. Statistical experts responsible for randomization designed the randomization parameters in advance, allowing the system to generate a random allocation table. The main clinical trial centers conduct eligibility screening for potential participants. Once deemed eligible, the researchers at each sub-center access the server via the internet and enter the information of the enrolled patients. The system then assigns a corresponding randomization number based on the random allocation table, determining the patient's placement in the respective study group.

Participants and recruitment

Patients will be recruited by radiation oncologists from each participating research center. For each interested patient, the clinician or clinical coordinator will provide a complete and comprehensive introduction to them or their designated representative, informing the patient about their rights, the risks involved, and the potential benefits they may receive to enhance their compliance with the protocol. Prior to enrollment, patients are required to sign an informed consent form, which will be kept in the Case Report Form (CRF). Patient registration is scheduled to begin on April 1, 2023, and

is expected to continue for 5 years (tentatively until January 2028). The final collection of data for the primary outcome measures is anticipated to be completed by December 2032.

Patient and public involvement

Neither patients nor the public were involved in the design, conduct, reporting, or dissemination plans of this study.

Objectives and endpoints

This study aims to evaluate whether adjuvant radiotherapy to the regional lymph nodes after breast-conserving surgery or chest wall plus regional lymph node radiotherapy after total mastectomy can further improve clinical outcomes in N1 patients with low clinical risk but high RecurIndex LRR risk.

The primary endpoint is invasive disease-free survival (IDFS). Secondary endpoints are any first recurrence (AFR), local-regional recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), recurrence-free survival (RFS), overall Survival (OS), disease-free survival (DFS), breast cancer-specific mortality (BCSM) and patient quality of life assessment. The specific definitions can be found in Table 2.

During the screening period and 3 months after the end of treatment, patients in each group fill out the quality of life questionnaire (EORTC QLQ-C30), as well as the breast cancer survival quality scale (EORTC QLQ-BS23) annually during the follow-up phase. (Supplementary 1 and 2).

RecurIndex Test

RecurIndex testing was performed using postoperative paraffin-embedded tissue sections from the primary lesion of the subjects. All sections were uniformly sent to the Jiangsu Simcere Pharmaceutical Co., Ltd., Jiangsu Simcere Diagnostics Co., Ltd. Formalin-fixed, paraffin-embedded tissue blocks should be selected that cover the largest amount of tumor cells and meet the diagnostic criteria in appearance. Tissue sections with an excess amount of normal tissue, necrotic tissue, adipose tissue, or hemorrhagic tissue should not be sent for examination. The tumor cell content in the identified sections should be >50% for the test to be performed. A total of 10 consecutive sections are needed, each with a thickness of 5 microns. The sections can remain unstained and without coverslips. There is no need to oven-dry the sections; they can be air-dried naturally.

Safety Assessment Indicators

All patients participating in the RIGAIN study are required to undergo safety assessments, including acute radiation reactions and late radiation injuries for radiotherapy patients. The evaluation criteria and handling of injuries are detailed in Supplementary 4-8. Their treatment is shown in Supplementar 9. Including acute skin reactions to radiotherapy, symptomatic radiation pneumonitis, long-term cosmetic outcomes (BCS/reconstruction patients), skin fibrosis (total mastectomy patients), ischemic heart disease, upper limb edema[27,28], brachial plexus injury and second primary tumor[29].

Radiotherapy

General consideration

The overall treatment plan for each participant is determined by the researchers at the corresponding

sub-center based on the participant's condition. Depending on their assigned group, patients will either undergo RNI or be exempted from it. Breast-conserving patients will all receive WBI. Patients should start radiotherapy within 8 weeks after completion of adjuvant chemotherapy. The regional lymph nodes include the supraclavicular lymph nodes and infraclavicular lymph nodes (unresected levels II/III axillary lymph nodes), with or without internal mammary lymph nodes (at least from the 1st to the 3rd intercostal space). For patients with minimally positive SLN and without ALND, the inclusion criteria encompass the low/intermediate axillary lymph nodes. The planned endocrine therapy and anti-HER2 treatment can be continued during the RT process.

Patient positioning and immobilization

The patient lies on a fixed device such as a breast support, vacuum bag, or foam pad. A CT scan is performed with a thickness of 3-5 mm, from the second cervical vertebra to the second lumbar vertebra. CT positioning includes surface marking, where lead wires are placed on the surgical scar of the primary lesion in breast-conserving patients or the chest wall scar in total mastectomy patients, as well as on the scar of the axillary sentinel/clearance lymph node incision. If there is a drainage site, it should also be separately marked with a lead wire or lead point.

Volumes of interest

The clinical target volume (CTV) and organs at risk (OAR) must be delineated on all CT slices, following the contouring guidelines of the Radiation Therapy Oncology Group (RTOG) and considering the actual situation at each CT slice. Detailed descriptions of CTV and OARs can be found in Supplementary 10. The margin between the planning target volume (PTV) and CTV depends on the institutional standards of each participating center, with a recommended minimum of 5 mm. Contours should be drawn according to the RTOG guidelines, including the ipsilateral and contralateral lungs, heart, humeral heads, and spinal cord.

External beam equipment and techniques

Radiation therapy techniques that can be employed include three-dimensional conformal radiotherapy (3DCRT), forward intensity-modulated radiotherapy (F-IMRT), inward intensity-modulated radiotherapy (I-IMRT), volumetric modulated arc therapy (VMAT), and helical tomotherapy (HT). Conventional radiotherapy (using a simulator for positioning and a 2D planning system to design treatment plans with external and tangential fields) and proton therapy techniques are not allowed. Some variations in treatment planning and implementation are permitted to accommodate the participating centers in adapting to the research protocol. However, it is strongly recommended that the treatment plans for enrolled patients at each center remain consistent to avoid confusion.

Dose prescription, fractionation

The whole breast target volume, or the integrated target volume of the whole breast and low to moderate axillary region, or the chest wall target volume, and the regional lymph node target volume receive a radiation dose of 5000 cGy in 25 fractions, delivered at a rate of 200 cGy per day, five days per week. Alternatively, a hypofractionated radiotherapy scheme can be chosen, with a radiation dose of 4000-4256 cGy in 15 to 16 fractions. For breast-conserving patients, a sequential tumor bed boost is performed after completion of whole breast irradiation, as determined by

individual center investigators. It can be delivered using conventional fractionation, with a dose of 1000 cGy in 5 fractions at a rate of 200 cGy per day, or by using hypofractionation, with a dose of 798 cGy-1064 cGy in 3 to 4 fractions at a rate of 266 cGy per day. If there are high-risk factors for local recurrence, such as positive surgical margins, close margins, or young age, the radiation dose for the tumor bed boost may be increased to 1400-1600 cGy in 7 to 8 fractions at a rate of 200 cGy per day.

DVH constraints

It is required that at least 95% of the prescribed dose to the PTV covers 95% of the PTV. The specific dose distribution is determined by each center's policy, with a recommended level as shown in Supplementary 11. For breast-conserving patients, it is recommended to achieve a central axis dose uniformity of ≤±7% for PTV_2 (whole breast or integrated target volume of whole breast and low to moderate axillary region) and PTV_1 (tumor bed), and to minimize the volume receiving ≥105% of the prescribed dose. The constraints for organs at risk should follow the Quantitative Analysis of Normal Tissue Effects in Clinical (QUANTEC) guidelines (see Supplementary 12).

Withdrawal from research and study termination

Termination of treatment

Research treatment will be terminated if any of the following conditions occur in the patient. The following are the criteria for the withdrawal or dropout of subjects:

1. The subject withdraws informed consent;
2. Any AE causes the subject to be unable to continue participating in the study;
3. The subject is lost to follow-up;
4. The subject does not comply with the study requirements and/or the investigator's instructions;
5. The subject has a concomitant illness or change in the subject's condition, and the investigator believes the subject is no longer suitable for the study treatment; or
6. For any other reason the investigator believes the subject is not suitable for continuing in the study.
7. If a subject drops out or withdraws, relevant safety and efficacy evaluations should be completed as soon as possible.

Study Termination

The trial will be terminated if any of the following situations occur during the trial:

1. Serious safety issues arise during the trial;
2. There is a major error in the study protocol;
3. The principal investigator voluntarily stops the trial; or
4. The administrative authority revokes the trial.
5. The termination of the trial may be temporary or permanent.

If the trial is terminated, all trial records should be retained for review.

Follow-up evaluation and toxicity assessment

The registration timeline, intervention measures, and assessments are presented in Table 3below.

In the follow-up phase after radiotherapy, check-ups and assessments will be performed every six months until the occurrence of an endpoint event or the end of the study. For patients without postoperative radiotherapy, check-ups and assessments will be conducted every six months after the completion of adjuvant chemotherapy until the occurrence of an endpoint event or the end of the

study. Effectiveness evaluations include tumor imaging examinations and assessments, brain MRI or CT, bone scans, quality of life questionnaires (EORTC QLQ-C30), and breast cancer-specific quality of life scales (EORTC QLQ-BS23). Safety evaluations include but are not limited to physical examinations, ECOG PS scores, pregnancy test checks, blood routine tests, blood biochemistry tests, adverse events, and serious adverse events. At the end of the study, participants will undergo physical examinations, performance status assessments (ECOG PS), blood routine tests, blood biochemistry tests, tumor markers, breast ultrasound/MRI, mammography, chest X-ray/CT, abdominal ultrasound/CT, and EORTC QLQ-C30 and EORTC QLQ-BS23 scoring. Evaluations of concomitant medications and adverse events are also required. The end-of-study visit window is 60 months after the last participant completes radiotherapy. For participants who are withdrawn or drop out before the end of the study, safety and effectiveness assessments will be conducted according to the requirements of end-of-study safety and effectiveness visits.

Data Management and Quality Assurance

In this study, electronic case report forms (eCRF) are used to collect data, and the EDC system designated by the principal investigator is used to complete the eCRF. Monitors verify the original data to ensure that the data entered into the eCRF by authorized trial center personnel (i.e., original data) is accurate, complete, and derived from original documents. Researchers and trial institutions must provide monitors with direct access to applicable source documents and reports for inspection and IEB/EC review. A Data Safety Committee has also been established, consisting of 5 members who are independent of the project team and have signed a research confidentiality agreement. The main tasks of the committee are to review and analyze positive results (recurrence and metastasis of subjects) and to understand the actual research results (without statistical analysis) when half of the subjects are enrolled. The committee will vote on whether it is necessary to adjust the research plan. This protocol does not include investigational drugs, and any toxicities that occur during treatment should be reported to the principal investigator and their ethics committee. In addition, the sub-centers should also report to the ethics committee of their institution. All serious adverse events and other adverse events must be recorded in the case report form.

Sample Size Estimation

This study is designed for superiority, referring to authoritative postoperative radiotherapy studies MA20[10] and EROTC22922[11] for N1 patients, in which the 5-year IDFS in the radiotherapy group and the control group were 90.7% vs 81.9% and 87.7% vs 77.1%, respectively. In the domestic RecurIndex external validation retrospective study[21] for N1 breast cancer patients, the 5-year IDFS in the high RI-LRR risk group was 81.1% vs 69.7% in the postoperative radiotherapy group and the control group, respectively. It is expected that the 5-year IDFS for the clinically low LRR risk and high RecurIndex LRR risk population in the experimental group and control group in this study will be 89% and 82%, respectively. The superiority margin is set to improve the primary endpoint IDFS by ≥7% (HR=0.587) in the postoperative radiotherapy research group compared to the control group. With a one-sided significance level (α) of 0.025 and a power (1-β) of 0.8, assuming the experimental group performs better than the control group, the required sample size for each group was calculated as 216 cases per group using PASS15.0 software. The allocation ratio between the experimental and control groups was set at 1:1. Considering a 5-year enrollment period, 5-year follow-up period, and potential 20% dropout rate (mainly considering the need for further 10-year and 15-year long-term

efficacy follow-up after reaching the 5-year endpoint), each group will need 270 cases, totaling 540 cases.

Statistical analysis

Descriptive statistical analyses will be conducted based on demographic characteristics such as age, gender, height, weight, and other baseline characteristics such as medical history. The Cox proportional-hazards model will be used for analysis of the primary endpoint. The Hazards Ratio and its 95% confidence interval will be calculated, including stratification factors and other covariates. Additionally, the Cox proportional-hazards model without covariates will be used to support the analysis results of the primary endpoint. Moreover, the Kaplan-Meier (KM) method will be used to calculate the median invasive disease-free survival for the two groups (experimental group vs control group), including 95% confidence intervals. KM plots will be used to illustrate the time trends of IDFS. For the secondary endpoints, the AFR of each group will be calculated, as well as the corresponding 95% Clopper-Pearson confidence intervals. LRFS, DMFS, RFS, OS, DFS, and BCSM will be analyzed for median values using the KM method (including 95% confidence intervals). The overall changes in EORTC QLQ-C30 and EORTC QLQ-BS23 scores from baseline will be summarized. Safety analysis will be conducted by summarizing adverse events, changes in laboratory test results, changes in vital signs, and study treatment exposure. The results will be reported by treatment group. All adverse events during treatment, grade 3 or higher TEAEs, serious adverse events (SAEs), radiotherapy-related SAEs, and TEAEs leading to study termination will be summarized by organ system, preferred term, and group in terms of numbers and percentages. A p value ≤ 0.05 in a two-tailed test will be considered statistically significant. Statistical analyses will be performed using SPSS V.25.0 (Statistical Package for Social Sciences) and STATA V.14.

Ethics and dissemination

This study has obtained approval from the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-097-02), as well as approval from the respective participating centers' ethics committees. The study is being conducted in accordance with the Helsinki Declaration and good clinical practice. Approval from the Chinese Human Genetic Resources Office was obtained on January 6, 2021, with the reference number 2020SQCJ2358. The study was registered on ClinicalTrials.gov on July 7, 2022, with the registration number NCT04069884. The research findings will be published in peer-reviewed journals. The authors will be individuals who have made significant contributions to the study, design, and implementation.

Confidentiality and protection of participants' rights and interests

Researchers are required to explain to participants that participation in the clinical trial is voluntary, and that they have the right to withdraw from the study at any stage without affecting their medical treatment and rights. Personal information of participants will be kept confidential. Participants should be informed about the nature, purpose, potential benefits, and possible risks of the clinical trial, as well as alternative treatment options. Researchers should ensure that the rights and obligations of participants, as stipulated in the declaration, are protected. Participants should be given adequate time to consider whether to participate and to sign the informed consent form.

Discussion

The RIGAIN study is a multicenter, open-label, randomized controlled phase III clinical trial. Our objective is to precisely assess patients with clinically low LRR risk N1 breast cancer who, if identified as high-risk for LRR by the RecurIndex test, may receive enhanced clinical efficacy from active RNI after BCS or CWI with RNI after mastectomy. The aim is to accurately identify patients who would benefit from intensified radiotherapy. In addition, we have established an observational study to investigate the potential to exclude RNI for patients who are clinically low LRR risk and are identified as low risk for LRR by the RecurIndex. The primary endpoint of the study is LRR, with the aim of identifying truly low-risk patients for whom radiotherapy can be safely omitted from planned treatment regimens. Similarly, the MA39 study defines a clinically and genetically low-risk LRR N1 population (age ≥40 years, luminal A type, Oncotype DX score <18) based on comprehensive clinical pathology, molecular subtype, and a multigene model. The anticipated results from the MA39 study could potentially guide personalized RNI decisions for patients who are found to be both clinically and genetically low risk. However, this study also has limitations. Firstly, the multi-gene models used in this study were developed to predict the risk of distant metastasis, and there may be inconsistencies between the occurrence of LRR and the risk of distant metastasis in clinical patients. Secondly, future research results are primarily intended to guide the omission of postoperative radiotherapy in clinically low-risk and genomically low-risk N1 patients. However, the significance of postoperative radiotherapy in patients with intersecting risks, particularly those who are clinically low-risk but genomically high-risk, remains unclear. Lastly, this study does not provide direct evidence for the application of Oncotype DX in guiding treatment decisions for Asian patients.

Traditionally, postoperative radiotherapy has been lauded for decreasing LRR and for helping to diminish the risk of distant metastases[10,11]. This has lead to long-term improvements in DFS and BCSS, providing the ultimate benefit to patients. Notably, this is attributed to the radiation-induced abscopal killing effect (RIAKE), which refers to a series of immunological responses induced by local high-dose radiotherapy that culminate in the elimination of tumors distant from the irradiation site[27]. In the context of postoperative radiotherapy for patients with regionally lymph node-positive breast cancer, the abscopal effect is most pronounced in patients classified as pN1, where the survival benefit is most conspicuous[9]. Compared to mastectomy, BCS better preserves the immune microenvironment, thereby enhancing the transformation and activation of the immune response following postoperative radiotherapy. This is the primary reason for our selection of IDFS as the main endpoint in this study.

Oncotype DX and MammaPrint assays primarily assess the overall recurrence risk and mainly guide chemotherapy and endocrine treatment[16,18,30]. Previous studies have indicated a high concordance in predicting the risk of distant metastases between the RecurIndex and the MammaPrint and Oncotype DX assays. However, some discrepancies exist in assessing the risk of LRR. The TAILORx study indicated that the RecurIndex predictive model may identify patients at risk of locoregional recurrence more accurately than the Oncotype DX[16,31,32].

The RecurIndex predictive model stands out amongst various multigene prediction models in early-stage breast cancer with the following unique characteristics and advantages: 1. Unlike other models that only assess overall recurrence risk and are more biased towards the risk of distant metastases,

RecurIndex can independently assess both the risk of locoregional recurrence and distant metastases, making it more suitable to guide precision radiotherapy; 2. RecurIndex demonstrates predictive efficacy in populations with HER2 overexpression and triple-negative breast cancer, potentially serving as a precise predictor of locoregional recurrence risk in patients with these two types of N1mic tumors, which could help guide individualized radiotherapy decisions.

The study focuses on the RecurIndex risk prediction model with the aim of guiding postoperative individualized radiotherapy for pT1-2N1M0 breast cancer patients. Particular attention is paid to the "clinically low LRR risk" but "genetically high-risk" population to explore and validate the clinical benefits of postoperative radiotherapy. The study design stands out for its clinical applicability and innovation as well as strict adherence to ethical and clinical practice standards. It effectively addresses the research gap in precise radiotherapy for N1 patients with overlapping risk profiles, both domestically and internationally. The study could potentially revolutionize the practice of postoperative radiotherapy by transitioning from a discretionary approach solely based on clinical and pathological information to an individualized optimization guided by clinical-genetic risk.

We anticipate that the RIGAIN study will generate high-quality evidence, establishing a precise risk assessment framework to guide optimized radiotherapy decisions for N1 breast cancer patients.

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Contributors

XH, YT and ZB designed the original protocol for the study. JC contributed to study management. JL, XH, YT and ZB drafted the manuscript.JL submitted the study. YT and ZB performed the sample size calculation and data analysis. RD and FW offer genetic testing. XH, JL, YT, JC, SH, AZ, LZ, YW, ZL, HY, XX, JC, XW-L, XL, XZ, WZ and XY participated in enrollment, treatment and follow-up of patients.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

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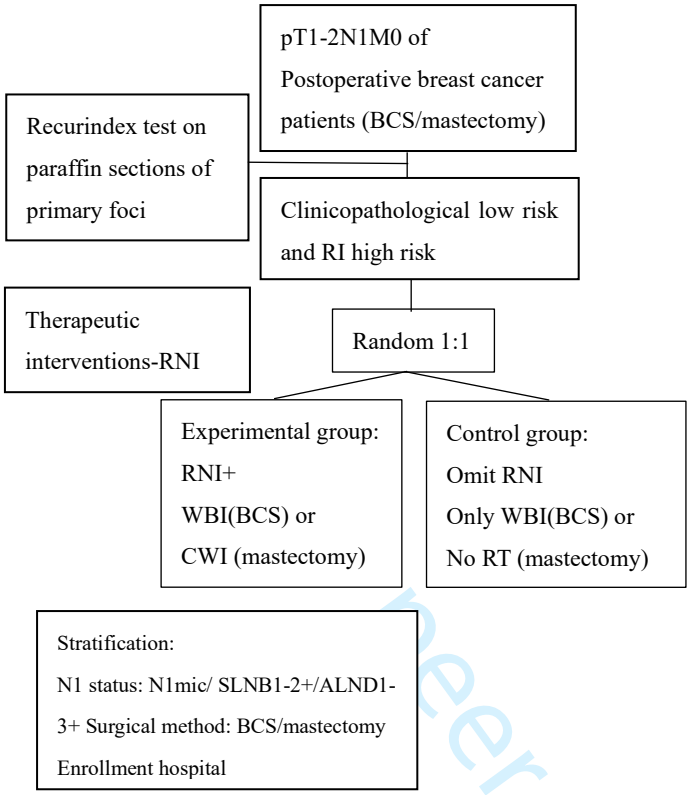

Figure 1 Research Process

Abbreviation: BCS, Breast conservative surgery; RI, Recurrence Index; RNI, Regional lymph node irradiation; WBI, Whole breast irradiation; CWI, Chest wall irradiation; RT, Radiotherapy; SLNB, Sentinel lymph node biopsy; ALND, Axillary lymph node dissection

Table1. Eligibility criteria for the study

Inclusion criteria
1. Age ≥18 years, ≤70 years;
2. ECOG PS ≤2 (Supplementary 3);
3. Postoperative pathology confirms the diagnosis of invasive breast cancer;
4. Meets the clinical definition of low risk: ① Axillary lymph node micrometastasis (N1mic), or ② N1 patients who meet all of the following conditions: a) Age ≥40 years; b) Lymphovascular invasion (LVI) negative or limited to individual or small foci of LVI (excluding extensive or large amounts of LVI); c) Three clinical molecular subtypes (Luminal A type, Luminal B1 type, and Luminal B2 type) are allowed in this study: ER-positive (ER≥1%) and HER2-negative, or ER-positive (ER≥1%) and HER2 overexpressing, respectively.
5. Postoperative pathological diagnosis of axillary lymph node status as any of the following: a. Sentinel lymph node biopsy or axillary lymph node dissection with micrometastasis (N1mic), b. Sentinel lymph node biopsy with 1-2 lymph node macrometastasis (N1sln), c. Sentinel lymph node biopsy + axillary lymph node dissection or simple axillary lymph node dissection with 1-3 lymph node metastasis (N1);
6. The primary tumor and breast underwent breast-conserving surgery or mastectomy ± breast reconstruction (autologous/prosthetic);
7. A thorough systemic examination (e.g., chest X-ray, ultrasound, CT, etc.) within 3 months before randomization for radiotherapy must confirm no distant metastasis;
8. Mammography and/or MRI within 12 months before surgery or randomization for radiotherapy must confirm no contralateral breast cancer;
9. Postoperative completion of at least 4 cycles of adjuvant chemotherapy containing anthracycline or taxane regimens;
10. Radiotherapy must be performed sequentially after the completion of all adjuvant chemotherapy, starting no later than 8 weeks after the end of chemotherapy;
11. Patients must have sufficient postoperative paraffin tissue sections of the primary tumor for RecurIndex testing;
12. No history of other malignant tumors, except for basal cell carcinoma of the skin; and
13. Signed informed consent before the start of the study.
Exclusion criteria
1. Confirmed T3-4, N0, N2-3, M1 stage disease before postoperative radiotherapy enrollment;
2. Received any neoadjuvant treatment before surgery, including chemotherapy, endocrine therapy, targeted therapy, or radiotherapy;
3. Patients who underwent mastectomy and only had sentinel lymph node biopsy;
4. History of contralateral breast cancer or other second primary malignant tumor (excluding basal cell carcinoma of the skin and cervical carcinoma in situ);
5. Previous history of chest radiotherapy;
6. Presence of severe heart, lung, liver, kidney, hematopoietic system, or nervous system diseases, or mental disorders;
7. Presence of scleroderma or active systemic lupus erythematosus or other autoimmune diseases;
8. Pregnant and breastfeeding patients.

Table 2. The specific definitions of the study endpoints

IDFS	The time from the day the subject is randomized to the earliest occurrence of invasive cancer local recurrence, distant metastasis, or death, but does not include contralateral breast second primary cancer.
AFR	Any ipsilateral chest wall, breast, regional lymph node recurrence, or distant metastasis event that occurs during the follow-up period.
LRFS	The time from the day the subject is randomized to the earliest occurrence of ipsilateral chest wall, breast, or regional lymph node recurrence or death.
DMFS	The time from the day the subject is randomized to the earliest occurrence of distant metastasis or death.
RFS	The time from the day the subject is randomized to the earliest occurrence of ipsilateral chest wall, breast, regional lymph node recurrence, distant metastasis, or death.
OS	The time from the day the subject is randomized until the patient's death.
DFS	The time from the day the subject is randomized to the recurrence of the disease or the patient's death due to disease progression.
BCSM	The time from the day the subject is randomized to death from breast cancer.

Table3. Research Schedule

Research Phase	Screening	Radiotherapy period				Follow up period										
Visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Time	-3M-0	1W	2W	3W	4-7W	6M	12M	18M	24M	30M	36M	42M	48M	54M	60M	In the event of a relapse
Informed consent	×															
Inclusion/exclusion criteria	×															
Frozen/paraffin tissue	×															
Pathology	×*															
Tumor markers	×					×	×	×	×	×	×	×	×	×	×	×
Breast + LN ultrasound/MR	×					×	×	×	×	×	×	×	×	×	×	
Mammography	×*						×		×		×		×		×	
Chest X-ray/CT	×					×	×	×	×	×	×	×	×	×	×	×
Abdominal ultrasound/CT	×					×	×	×	×	×	×	×	×	×	×	×
Thyroid + LN ultrasound	×					×	×	×	×	×	×	×	×	×	×	
Bone Scan	×*						×		×		×		×		×	×
Cranial CT/MRI																×***
Demographic features	×															
Medical history	×															
Physical examination	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	

Upper and lower arm circumference measurement◆	×				×		×		×		×		×		
Body weight	×				×		×		×		×		×		
ECOG score	×				×		×		×		×		×		
Blood count	×**	×	×	×	×	×	×	×	×	×	×		×	×	×	×
Liver and kidney function	×**				×	×	×	×	×	×	×		×	×	×	×
Infectious disease	×*															
Serum pregnancy test	×**															
EORTC QLQ-C30	×**				×		×		×		×		×		×	
EORTC QLQ-BR23	×**				×		×		×		×		×		×	
Harris	×**						×		×		×		×		×	
Baker	×**						×		×		×		×		×	
CTCAE V4.03	×**	×	×	×	×		×	×	×		×		×		×	
Acute radiation reaction		×	×	×	×											
Late radiation injury							×	×	×		×		×		×	

◆Upper and lower arm circumference measurements: measured at the bilateral metacarpophalangeal joints (mid-metacarpal), at the wrist, and at the distal 10 cm and proximal 15 cm of the elbow epicondyle.

* Within 12 months prior to randomization

** Within 14 days before randomization

*** When cranial symptoms were present

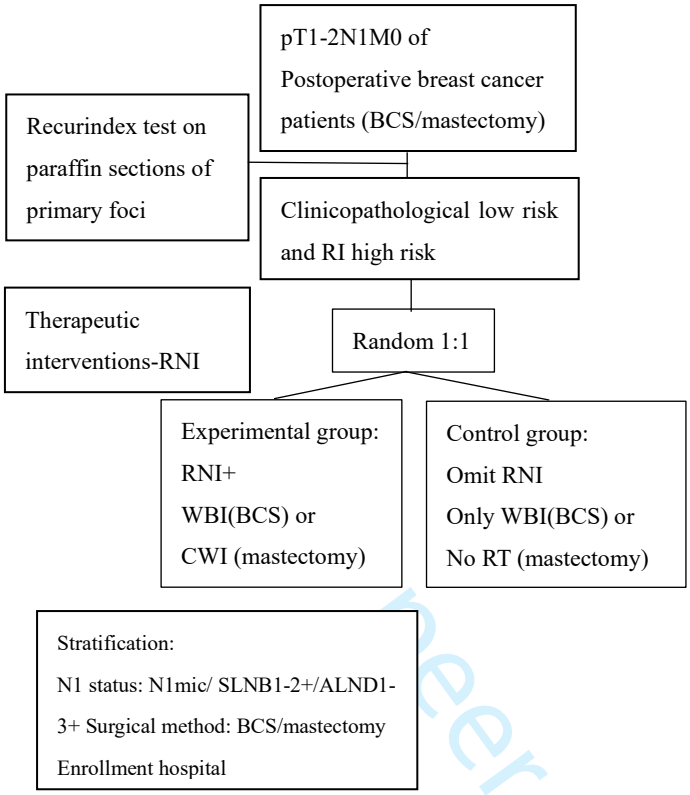

Figure 1 Research Process

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Spplementary 1. Quality of Life Questionnaire EORTC QLQ-C30 (version 3)

We are interested in learning some information about you and your health status. Please answer all of the following questions independently and circle the answer that is most appropriate for you. There are no "correct" or "incorrect" answers. The information you provide will be kept strictly confidential.

Please fill in your last name: _____

Date of birth (year, month, day): _____

Today's date (year, month, day): _____

	No	A little	Some	Very much
1.Do you feel difficulty when you do some laborious movements, such as lifting heavy shopping bags or luggage?	1	2	3	4
2. Do you find it difficult to walk long distances?	1	2	3	4
3. Do you find it difficult to walk short distances outdoors?	1	2	3	4
4. During the day, do you have to lie in bed or sit in a chair?	1	2	3	4
5. Do you need assistance with eating, dressing, washing or going to the bathroom?	1	2	3	4
In the past week:	1	2	3	4
6. Are your work or daily activities limited by physical ability?	1	2	3	4
7. Are your hobbies and leisure activities physically limited?	1	2	3	4
8. Do you ever feel short of breath?	1	2	3	4
9. Have you ever had any pain?	1	2	3	4
10. Have you ever needed rest?	1	2	3	4
11. Have you ever felt sleep deprived?	1	2	3	4
12. Have you ever felt weak?	1	2	3	4
13. Have you ever felt a lack of appetite?	1	2	3	4
14. Have you ever felt nauseous and wanted to vomit?	1	2	3	4
15. Have you ever vomited?	1	2	3	4
16. Have you ever had constipation?	1	2	3	4
17. Have you ever had diarrhea?	1	2	3	4
18. Do you ever feel tired?	1	2	3	4
19. Does pain interfere with your daily activities?	1	2	3	4
20. Do you have difficulty concentrating on things, such as reading the newspaper or watching TV?	1	2	3	4
21. Do you ever feel nervous?	1	2	3	4
22. Do you ever feel worried?	1	2	3	4
23. Do you ever feel easily irritated?	1	2	3	4
24. Do you ever feel depressed?	1	2	3	4
25. Do you ever have trouble remembering things?	1	2	3	4
26. Has your medical condition or treatment process interfered with your family life?	1	2	3	4
27. Has your medical condition or treatment interfered with your social activities?	1	2	3	4
28. Has your medical condition or treatment process caused you financial difficulties?	1	2	3	4

For the following questions, the numbers 1-7 represent a scale from "very poor" to "very good".

29. How would you rate your overall health in the past week?

1 2 3 4 5 6 7

very poor" to very good

30. How would you rate the overall quality of your life in the past week?

1 2 3 4 5 6 7

very poor" to very good

Patients sometimes have the following clinical symptoms. Please indicate the extent of these clinical symptoms or problems you have had in the past week, circling the answer that best applies to you.

	No	A little	Some	Very much
31. Do you cough a lot?	1	2	3	4
32. Do you cough up blood (blood in sputum)?	1	2	3	4
33. Do you feel short of breath when you rest?	1	2	3	4
34. Do you feel short of breath when you take a walk?	1	2	3	4
35. Do you feel short of breath when climbing stairs?	1	2	3	4
36. Have you ever had pain in your mouth or tongue?	1	2	3	4
37. Have you ever had difficulty swallowing?	1	2	3	4
38. Have you ever had tingling/numbness in your hands and feet?	1	2	3	4
39. Have you ever had hair loss?	1	2	3	4
40. Have you ever had chest pains?	1	2	3	4
41. Have you ever had pain in your arms or shoulders?	1	2	3	4
42. Have you ever had any pain in other parts of your body?	1	2	3	4
If yes, please write down the area:				
43. Have you ever taken any painkillers?				
1.Yes 2.No				
If you have used it, does it help much with pain?	1	2	3	4

Supplementary 2. Breast Cancer Survival Quality Scale EORTC QLQ-BR23

Please recall if you have experienced any of these symptoms or the extent of the problem and tick the appropriate number"√"

In the past 1 week	No	A little	More	A lot
1. Do you have dry mouth?	1	2	3	4
2. Do your food and drinks taste different than usual?	1	2	3	4
3. Do your eyes hurt, feel uncomfortable, or tear up?	1	2	3	4
4. Do you have hair loss?	1	2	3	4
5. If you have hair loss, does it bother you?	1	2	3	4
6. Do you feel sick or uncomfortable?	1	2	3	4
7. Is your face red and hot?	1	2	3	4
8. Do you have a headache?	1	2	3	4
9. Do you feel less physically attractive due to illness or treatment?	1	2	3	4
10. Do you feel less attractive as a woman due to illness or treatment?	1	2	3	4
11. Do you have difficulty looking at your naked body?	1	2	3	4
12. Are you dissatisfied with your body?	1	2	3	4
13. Are you worried about your future health?	1	2	3	4

In the past 4 week				
14. How interested are you in sex?	1	2	3	4
15. How active are you sexually (do you have sex often)? (With or without sex?)	1	2	3	4
16. If you have sex, to what extent does it bring you pleasure?	1	2	3	4

In the past 1 week
17. Do you have pain in your arm or shoulder?
18. Is your arm or hand swollen?
19. Do you have difficulty lifting or moving your arm to the side?
20. Do you have pain in the area of your affected breast?
21. Is the area of your affected breast swollen?
22. Do you have hypersensitivity in the affected breast area?
23.Do you have skin problems (e.g. itching, dryness, flaking) in the affected breast area?

Supplementary 3. Physical status ECOG scoring criteria

ECOG scoring criteria	Scoring
Mobility is completely normal and does not differ in any way from that before the onset of the disease	0
Can walk freely and perform light physical activities, including general housework or office work, but cannot perform heavier physical activities	1
Able to walk freely and take care of themselves, but have lost the ability to work, and can get up and move around at least half of the time during the day	2
Only partially able to take care of themselves, bedridden or wheelchair bound for more than half of the day	3
Bedridden and unable to care for themselves	4
Death	5

Supplementary 4. Evaluation criteria for common adverse events (CTCAE Version 4.03)
(excerpt, normal common adverse event evaluation criteria is grade 0)

Adverse Events	Grading				
	1	2	3	4	5
Hemoglobin g/L	Normal value -10.0	10.0-8.0	8.0-6.5	<6.5	
Leukocytes(10^9/L)	Normal value-3.0	3.0-2.0	2.0-1.0	<1.0	
Neutrophils(10^9/L)	Normal value-1.5	1.5-1.0	1.0-0.5	<0.5	
Platelets(10^9/L)	Normal value-75	75-50	50-25	<25	
Transaminase ALT/AST	≤2.5×N	2.6-5.0×N	5.1-20×N	>20×N	
Alkaline phosphatase	≤2.5×N	2.6-5.0×N	5.1-20×N	>20×N	
Bilirubin	ULN-1.5×N	1.5-3.0×N	3.0-10×N	>10×N	
Creatinine Cr	ULN-1.5×N	1.5-3.0×N	3.0-10×N	>10×N	
Weight gain/loss	5.0-10%	10-20%	≥20%		
Vomiting	Vomiting 1 time in 24h during treatment	Vomiting 2-5 times in 24h during treatment	Vomiting ≥ 6 times in 24h during treatment or requiring fluids	Life-threatening and requires urgent treatment	Death
Coughing sputum	Occasional/mild coughing of sputum	Moderate cough and sputum; interferes with instrumental daily life	Persistent heavy coughing and limited personal self-care		
Pneumonia	Asymptomatic; clinical examination or diagnostic findings only; no intervention required	Symptomatic (mild cough and/or dyspnea, with or without fever); requires clinical intervention; interferes with instrumental daily life	Severe symptoms; limited personal autonomy; need for oxygen	Life-threatening respiratory dysfunction; requiring urgent treatment (tracheotomy or intubation)	Death
Acute coronary syndrome		Symptomatic, progressive angina; normal cardiac enzymes; hemodynamically stable	Symptomatic, unstable angina with/ or acute myocardial infarction, abnormal cardiac enzymatic parameters, hemodynamically stable	Symptomatic, unstable angina with/ or acute myocardial infarction, abnormal cardiac enzymatic parameters, hemodynamic instability	Death

Left ventricular systolic insufficiency			Symptoms of decreased ejection fraction	Uncontrollable heart failure with declining ejection fraction requiring urgent intervention	Death
Heart Failure	Asymptomatic, with abnormalities detected by laboratory tests (e.g., natriuretic peptide) or cardiac imaging	Mild to moderate symptoms with activity or exercise	Symptoms occur at rest or with light activity or exercise; requires treatment	Life-threatening; requires urgent treatment (e.g. continuous infusion therapy, mechanically assisted circulation)	Death
Limb edema	Comparison using the greatest difference in volume or circumference, with 5% to 10% variation between limbs; edema or blurred anatomy that can only be detected on close examination	Comparison using the largest difference in volume or circumference, 10% <~30% difference between limbs; disappearance of skin folds; apparent loss of limb anatomy, change in shape; interferes with instrumental daily living	>30% volume variation between limbs; severe changes in limb shape; limited personal autonomy		
Neurotoxicity - Sensory	Mild sensory abnormalities (including paresthesia), absence of deep tendon reflexes	Moderate objective sensory deficit or sensory abnormalities (including tingling)	Severe objective sensory loss or sensory abnormalities that affect daily life	Persistent sensory loss, affecting function	Death
Neurotoxicity-motor	Self-perceived weakness with no objective findings	Moderate self-conscious weakness; no significant functional impairment	Self-perceived weakness with functional impairment	Paralysis	Death

ULN, Upper limit of normal value

Supplementary 5. Scoring criteria for acute radiation reactions (RTOG/EORTC 1995)

Organ Tissue	Grading				
	0	1	2	3	4
Skin	No change	Punctate skin erythema, alopecia, dry peeling or decreased sweating	Marked erythema, patchy wet peeling or moderate edema of the skin	Presence of fused wet desquamation or surgical edema	Skin ulcers, bleeding or necrosis
Larynx	No change	Mild to moderate hoarseness/cough without cough suppressants/mucosal edema	Persistent hoarseness but vocalization/involved otalgia, sore throat, flaky	soft speech, sore throat or involved otalgia requiring	Significant dyspnea, wheezing, hemoptysis requiring tracheotomy or intubation
Pharynx and esophagus	No change	Mild dysphagia requiring general analgesia or/non-narcotic analgesia/need for semi-liquid diet	Moderate dysphagia/narcotic analgesia/fluid	narcotics / fused fibrin exudate, marked arytenoid	Complete obstruction, ulceration ulceration, perforation, sinus tract
Lungs	No change	Mild symptoms, mild dry cough or exertional dyspnea, may be associated with imaging changes	Moderate symptoms, persistent cough requiring narcotic cough suppressant treatment or dyspnea with mild activity	Severe dysphagia dehydration or weight loss >15%, need for gastric feeding or intravenous fluids Severe cough or dyspnea at rest with severe symptoms, ineffective sedation or cough suppressants, clinical and imaging evidence of acute pneumonia, requiring intermittent oxygen or hormonal therapy	Severe respiratory insufficiency requiring continuous oxygen or assisted ventilation
Heart	No change	Asymptomatic but objective evidence of ECG changes or pericardial abnormalities, no evidence of other cardiac disease	Symptoms not requiring specific treatment with ECG changes and imaging changes of congestive heart failure or pericardial disease	Congestive heart failure, angina pectoris or pericardial disease for which drug therapy is effective	Congestive heart failure, angina pectoris, pericardial disease or arrhythmias that have not responded to non-surgical treatment

Supplementary 6. Scoring criteria for late radiation injury (RTOG/EORTC 1995)

Organ Tissue	Grading				
	0	1	2	3	4
Skin	No change	Mild atrophy, hyperpigmentation, partial hair loss	Lamellar atrophy, moderate capillary dilatation, total hair loss	Significant atrophy, marked capillary dilation	Ulcers
Subcutaneous tissue	No change	Mild sclerosis (fibrosis) and loss of subcutaneous adipose tissue	Moderate fibrosis but asymptomatic, slight constriction of irradiated field <10% of the side length	Severe sclerosis and loss of subcutaneous tissue. Constriction of the irradiated field >10% border length	Necrosis
Lungs	No change	Asymptomatic or mildly symptomatic (dry cough), mild imaging signs	Moderate symptomatic pulmonary fibrosis or pneumonia (severe cough), hypothermia, patchy imaging	Severe symptomatic pulmonary fibrosis or pneumonia with dense imaging changes	severe respiratory insufficiency requiring continuous oxygenation or assisted ventilation
Heart	No change	Asymptomatic or mildly symptomatic; temporary T-wave inversion and ST changes; sinus tachycardia >110 beats/min at rest	Moderate exertional angina; mild pericarditis; normal heart size; persistent T-wave abnormalities and ST changes; low QRS waves	Severe angina pectoris; pericardial effusion; constrictive pericarditis; moderate heart failure; cardiac enlargement; abnormal electrocardiogram	pericardial tamponade; severe heart failure; severe constrictive pericarditis

Supplementary 7. Harris Cosmetic Grade Rating of Cosmetic Breast Preservation/Reconstructive Surgery Results

Level	Double breast symmetry	Double nipple level gap	Breast shape on the affected side	Skin
Excellent, Good	Symmetries	≤2cm	No significant difference with the healthy side, normal appearance, no deformation of the breast lift due to scarring, no difference between the affected side and the healthy side in feel	Normal
General	Symmetries	2cm-3cm	The shape of the affected breast is basically normal or slightly smaller than the healthy side, and the feel of the affected side is slightly worse.	Lightened or shiny color
Bad	Obvious asymmetry	>3cm	The appearance of the affected side of the breast changes and is significantly smaller than the healthy side, and feels poorly in the hand	Thick, rubber-like, rough

Supplementary 8. Baker's classification of the prosthetic envelope

Grading	Breast Implants
I (no accessible envelope)	Breast implants feel as soft as non-operated breasts
II(Lightly hardened)	The softness of the breast is slightly worse, the implant can be touched but not seen
III(Heavy hardening)	Harder breasts, implants can be easily touched out or visible deformation of the implant
IV(severe contracture)	Breasts are hard, painful when touched, skin temperature becomes cold, deformation is obvious

Only Baker grade III and IV are defined as periosteal contracture and require reoperation

Supplementary 9. **Radiotherapy-related adverse reactions and their treatment**

1 Radiation-induced skin damage

Early skin reactions are those that occur within three months after the start of radiotherapy and are the most common complications in breast radiotherapy. Approximately 92% of patients receiving post-lumpectomy radiotherapy will experience acute radiation-induced skin reactions, mostly grade 1 or 2 mild reactions, with a wet desquamation incidence rate of about 3%. Patients undergoing mastectomy will almost always experience acute radiation-induced skin reactions, mostly grade 2 reactions, with a wet desquamation incidence rate of about 10-20%.

Prevention is the main approach to managing radiation-induced skin complications. For grade 1 and 2 injuries, conservative treatment is primarily used. Patients should wear loose, cotton open-front underwear, avoid friction and pressure on the skin in the irradiation area, avoid using irritating products such as soap and shower gel, avoid bathing with hot water or showering the irradiation area, and not apply chemical ointments or adhesive tape. If the skin is red, swollen, itchy, or painful, do not scratch it or apply medication randomly. Follow the doctor's advice for medication, such as triethanolamine cream, compound vitamin B12 solution, and medical radiation protectants. Wet dermatitis can be treated with exposure therapy, keeping the area dry and avoiding secondary infections. Wet dermatitis that does not heal after more than two months may develop into skin necrosis, often requiring surgical treatment, with skin grafting for larger areas.

Late skin reactions include local hyperpigmentation, telangiectasia, atrophy, and fibrosis. For chronic radiation dermatitis with recurrent ulceration and significant worsening, surgery is often used to prevent malignant transformation.

2 Pharyngeal and esophageal reactions

Irradiation of the supraclavicular area can cause pharyngeal pain and difficulty swallowing, which are generally mild and self-limiting. Prevention methods include using new radiotherapy techniques, accurate positioning, precise delineation of the target area, rational design of radiation fields, and reducing or avoiding irradiation of organs at risk. Symptomatic support treatment is provided for severe reactions.

3 Radiation-induced lung injury

Radiation-induced lung injury includes early radiation pneumonitis occurring within 3 months after radiotherapy and late radiation-induced pulmonary fibrosis occurring after 3 months. Approximately 2/3 of patients will develop asymptomatic radiation pneumonitis, which does not require treatment. The incidence of symptomatic radiation pneumonitis is between 1% and 5%, usually occurring within 2 months after radiotherapy or within 6 months after radiotherapy. Patients have symptoms and signs of pneumonia, which can manifest as cough, sputum, or fever, and in severe cases, dyspnea and hypoxia. In particular, when imaging examinations (chest X-rays and CT scans) show inflammatory exudative changes in the lung tissue within the irradiation field, symptomatic radiation pneumonitis can be diagnosed after excluding lung metastasis and tuberculosis. Supportive treatment, including hormones, oxygen therapy, and even mechanical ventilation, can provide complete relief, but some patients may still develop pulmonary fibrosis within 6-12 months, even with treatment. Pulmonary fibrosis is a late injury caused by damage to the lung interstitium and pleura, and in severe cases, it can be life-threatening.

There is currently no specific treatment for radiation pneumonitis, so prevention is more important than treatment. For patients undergoing whole-breast irradiation alone, it is recommended to use a dose-volume constraint of V20 < 22% for the ipsilateral lung. For those receiving irradiation of the supraclavicular lymph node region, a dose-volume constraint of V20 < 34% and V30 < 22% should be used for the ipsilateral lung to further evaluate the overall radiotherapy plan.

4 Radiation-induced heart damage

Radiation-induced heart disease (RIHD) initially manifests as acute pericarditis and later as coronary artery disease, chronic pericarditis, myocardial fibrosis, cardiomyopathy, heart valve damage, and cardiac conduction abnormalities. A 2013 New England Journal article reported that for every 1 Gy increase in the average dose to the heart, the incidence of major coronary events increased by 7.4%.

Reducing the risk of RIHD is also focused on prevention. The most fundamental measure is to minimize or avoid radiation exposure to the heart during radiotherapy. The Chinese Anti-Cancer Association Breast Cancer Diagnosis and Treatment Guidelines and Standards (2015 Edition) recommend that the average radiation dose to the heart should be assessed to be at least below 8 Gy. It is recommended to limit the heart's V30 to less than 10%. In addition, for high-risk populations of RIHD or those with cardiovascular

disease, drugs that have a protective effect on the cardiovascular system should be used as soon as possible. Regular cardiac ultrasound follow-ups should be conducted during the follow-up phase.

5 Upper limb edema

Edema in the affected upper limb is one of the common complications after breast cancer surgery and/or radiotherapy, and the extent of surgery is an important influencing factor. AMAROS research reported that the 1-year, 3-year, and 5-year lymphedema incidence rates for the ALND group were 28%, 23%, and 23%, respectively, significantly higher than the 15%, 14%, and 11% for the SLNB + axillary radiotherapy group. The incidence of upper limb lymphedema after axillary lymph node biopsy alone is 5%. Edema caused by radiotherapy usually occurs 1 to 2 months after the end of radiotherapy. Depending on the time of onset, upper limb edema can be divided into early edema and delayed edema occurring after several years. Upper limb edema caused by tumor recurrence in the axilla and supraclavicular region is not considered a true post-treatment complication.

The main prevention method for upper limb edema is to reduce axillary dissection, and postoperative progressive functional exercise is the key to preventing upper limb edema. When upper limb edema occurs, manual massage or compression therapy can be used.

6 Brachial plexus nerve injury

Radiation-induced brachial plexus nerve injury is a rare late complication after breast cancer radiotherapy, with an incidence rate of 1%-4%. Early symptoms include sensory and motor disorders in the affected limb and pain, often accompanied by severe nocturnal pain. Some cases may also have lymphedema, with progressively worsening functional impairment. In the late stage, this can lead to the loss of function of the entire limb, causing lifelong disability for the patient and severely affecting the patient's daily life and rest, with a significant impact on their mental health and quality of life. A preliminary diagnosis can be made based on the patient's radiotherapy history, asymptomatic intervals, and clinical features in clinical practice. However, it is necessary to rule out brachial plexus nerve injury caused by tumor metastasis or compression.

Radiation-induced brachial plexus nerve injury is irreversible, and there is currently no ideal treatment method, so prevention is crucial. It is essential to strictly follow the indications for radiotherapy in the lymphatic drainage area and pay attention to the radiotherapy range and radiation dose. For cases without severe pain, active measures should be taken to improve the blood supply of the nerves and surrounding soft tissues, and the earlier the diagnosis and treatment, the better the results. For advanced cases, treatment is aimed at relieving pain and improving quality of life.

7 Second primary tumors

Second primary tumors that can occur after breast cancer treatment include contralateral breast cancer and other malignant tumors such as lung cancer and soft tissue sarcomas. If these second primary tumors can be diagnosed and treated early, they do not affect the patient's survival. Therefore, regular follow-up of patients should be strengthened in clinical practice.

8 Rib fractures

The incidence is less than 1%. In most cases, patients have no noticeable symptoms, and fractures are discovered during bone scans or X-ray examinations. A small number of patients may experience chest wall or rib pain, which generally heals on its own without the need for special treatment.

9 Other side effects

During radiotherapy, patients may experience mild loss of appetite and fatigue. Therefore, it is important to adjust the diet reasonably, advocating for a "high protein, high vitamin, low fat" diet to maintain a balanced nutrition. Regularly review routine blood tests, and if a decrease in white blood cells is found, there is a risk of infection. In such cases, it may be necessary to temporarily pause radiotherapy and follow the doctor's advice for symptomatic supportive treatment.

Supplementary **10** Guidelines for Target Volume Delineation

Clinical practice involves the use of RNI+WBI(BCS)/CWI (complete mastectomy) in patients who are of low-risk clinically but high-risk in terms of RecurIndex LR.

1.1 Whole Breast Clinical Target Volume (CTV_2)

1. Purpose: Applicable for patients who undergo breast conservation surgery and axillary lymph node dissection.
2. Target definition: The entire or majority of the mammary gland on the affected side, encompassing the entire tumor bed target range, entire retro mammary space and pectoralis major fascia, Rotter's lymph nodes in the pectoralis major and minor inter-space, and the mid and lower axilla within the entire breast target range that was not cleared.
3. Target boundaries: • The upper boundary is the palpable/CT-visible upper edge of the gland. • The lower boundary is the palpable/CT-visible lower edge of the gland. • The anterior boundary is 5mm subcutaneously, or 0.3cm subcutaneously or even up to the skin for thin and small breasts. • The posterior boundary is 1-2mm behind the surface of the pectoralis major fascia (adjacent to the retro mammary space), not leaving a fatty gap, excluding ribs/intercostal muscles, but including Rotter's lymph nodes and axillary regions I and II if regional lymph nodes are metastatic. • The medial boundary is next to the sternum, at least to the inner edge of the peristernal vessels. • The lateral boundary is the palpable or CT-visible lateral edge of the gland, anterior to the thoracodorsal artery, anterior edge of the latissimus dorsi muscle.

1.2 Tumor Bed and CTV_1

1. Purpose: Applicable for patients undergoing breast-conserving surgery. Boost to the tumor bed is not mandatory and is determined by the policy of each center. Boost is suggested under the following circumstances: positive margin, close margin (invasive cancer or DCIS with a negative margin ≤2mm), and young age (<50 years).
2. Tumor bed definition: The range of the excised tumor and its surrounding tissue cavity, filled with serous effusion or formed tissue.
3. Tumor bed boundaries: Refer to: (1) The location of titanium clips, with a suggestion of placing clips at the left, right, top, bottom, and rear (2) The range of serous effusion, including that within the gland and sub-scar.
4. Tumor Bed CTV_1: Includes the mammary gland and soft tissues 10-15mm beyond the surgical excision of the tumor bed. It is suggested to reduce this for patients undergoing segmental resection to about 10mm. If there is no gland beyond the tumor bed, consider reducing it; for positive margins or presence of EIC or severe ADH, it is crucial to appropriately increase the range.

1.3 Integrated Target Area of the Whole Breast and Lower and Middle Axillary CTV_2

1. Purpose: Applicable for patients undergoing breast conservation surgery plus sentinel lymph node biopsy.
2. Target definition: Whole breast + axillary lymph nodes in zones I and II + Rotter's lymph nodes between pectoralis major and minor muscles.
3. Target boundaries: Whole breast boundaries refer to 5.2.3.1.1 Whole Breast Target; axillary zone I: Anatomically marked by the pectoralis minor muscle, located laterally to the pectoralis minor; axillary zone II: Follows the course of the axillary vein, located posterior to the pectoralis minor including the intermuscular space of pectoralis major and minor (Rotter's LN).

Criterion	Axillary Zone I	Axillary Zone II	Rotter's Lymph Nodes
Superior Boundary	Where the axillary vessels cross the lateral edge of the pectoralis minor muscle	Where the axillary vessels cross the medial edge of the pectoralis minor muscle	Including the cephalic side of the axillary artery and 5mm above the axillary vein
Inferior Boundary	Where the pectoralis major muscle inserts into the rib	Where the axillary vessels cross the lateral edge of the pectoralis minor muscle	Inferior boundary of axillary zone II
Anterior Boundary	Anterior to the pectoralis major muscle and latissimus dorsi muscle	Anterior to the pectoralis minor muscle	Posterior to the pectoralis major muscle
Posterior Boundary	Anterior to the subscapularis muscle	Rib and intercostal muscles	Anterior to the pectoralis minor muscle
Medial Boundary	Lateral edge of the pectoralis minor muscle	Medial edge of the pectoralis minor muscle	Medial edge of the pectoralis minor muscle
Lateral	Medial aspect of the latissimus	Lateral edge of the pectoralis	Lateral edge of the pectoralis

Boundary	dorsi muscle	minor muscle	minor muscle

1.4 Clinical Target Volume for Chest Wall (CTV_CW)

1. Purpose: Applicable to patients who have undergone total mastectomy ± breast reconstruction surgery.
2. Target Definition: The surgical area that may cause intraoperative spread and carry the risk of recurrence after total mastectomy.
3. Target Boundaries • Upper boundary is a clinical marker/0.5-1cm below the clavicle head; • Lower boundary is a clinical marker/below the contralateral breast fold; • Anterior boundary is the skin, excluding lead wire; • Posterior boundary is the intercostal muscle of the rib; • Medial boundary is a clinical marker/at the junction of the sternum and ribs; • Lateral boundary is a clinical marker/ at the anterior edge of the thoracic and dorsal vessels and the latissimus dorsi.
    - Note: ① Includes all scars, with the target area 2cm above and below the scar not reduced; ② Includes postoperative changes observed on CT scan (granuloma and fibrotic changes, barb-like muscle irritation signs).

1.5 Clinical Target Volume for Supraclavicular and Infraclavicular Lymph Nodes (CTV_LN)

1. Purpose: Suitable for group A patients with low clinical risk and high RecurIndex LR risk requiring regional lymph node radiation.
2. Target Definition: Includes supraclavicular area, neck IV region, and infraclavicular lymph nodes (unsurgically treated Level III axillary lymph nodes).
3. Target Boundaries • Upper boundary is below the cricoid cartilage; • Lower boundary is 0.5-1cm below the clavicle head, where the subclavian vein disappears, and connects with the whole breast/chest wall target area; • Anterior boundary is behind the sternocleidomastoid muscle above and behind the pectoralis major muscle below; • Posterior boundary is the posterior edge of the anterior scalene muscle above and the anterior edge of the rib and intercostal muscle below; • Medial boundary is the internal jugular vein above, including the entire scalene muscle gap to the level of the transverse cervical artery and vein, and the junction of the subclavian vein and internal jugular vein below; • Lateral boundary is the lateral edge of the sternocleidomastoid muscle above and the lateral edge of the pectoralis minor muscle below.
    - Note: ① Avoid the operated axillary area (Level I and part of Level II); ② Includes the unsurgically treated axillary Level II area.

1.6 Internal Mammary Lymph Node Area (CTV_IMN)

1. Purpose: Applicable to Group A patients with low clinical risk and high RecurIndex LR, requiring regional lymph node irradiation. Irradiation of the internal mammary lymph node region is not obligatory and is determined by the radiation oncologist.
2. Target area definition: Those at risk of internal mammary lymph node metastasis may benefit from internal mammary lymph node irradiation. It includes the 1st-3rd intercostal spaces, 5mm in all directions from the intrathoracic vessels, the upper boundary infusing into the supraclavicular area, and the lower boundary at the upper edge of the 4th rib.
3. Target area boundaries: • Superior border: Infusion into the supraclavicular area; • Inferior border: Upper edge of the fourth costal cartilage; • Anterior border: Back of the pectoralis major muscle, back of the sternum; • Posterior border: Pleura or 5mm behind the internal mammary vessels; • Medial border: 5mm inside the internal mammary vessels, including the whole space between the sternum and vessels; • Lateral border: 5mm outside the internal mammary vessels, outer edge of the brachiocephalic vessels.
    - Note: ① For high-risk patients, the upper boundary extends to the junction of the internal jugular vein, subclavian vein, brachiocephalic vein, and internal mammary vein; ② It is recommended to expand at least 5mm inside and outside the direction of the internal mammary vessels.

1.7 Intraclavicular Lymph Nodes (CTV_intraclavicular-LN)

1. Purpose: Applicable to Group A patients with low clinical risk and high RecurIndex LR, requiring regional lymph node irradiation. Irradiation of the intraclavicular lymph node area is not obligatory and is determined by the radiation oncologist.
2. Target area definition and boundaries: Those at risk of intraclavicular lymph node metastasis may benefit from intraclavicular lymph node irradiation. The upper boundary is the horizontal level of the transverse cervical artery, the lower boundary is the upper edge of the brachiocephalic trunk, the medial boundary is the midline of the body, and the lateral boundary is the inner edge of the supraclavicular area.

- Note: ① When irradiating the internal mammary lymph nodes, it should be routinely outlined; ② When there is metastasis of supraclavicular lymph nodes, it should be routinely outlined; ③ When there is extracapsular invasion of axillary Level II/III lymph nodes, it should be routinely outlined; ④ In patients with primary tumors invading the deep fascia, or the primary lesion located in the medial and upper part of the breast, consider outlining.

In patients who are clinically low-risk but exhibit a high-risk RecurIndex LR, regional nodal irradiation (RNI) is omitted, with whole breast irradiation (WBI) after breast-conserving surgery (BCS) only and no chest wall irradiation (CWI) after total mastectomy.

2.1 Entire Breast Target Volume (CTV_2):

1. Purpose: Suitable for patients who have undergone breast conservation surgery with axillary lymph node dissection.
2. Target Definition: This includes all or most of the glandular tissue of the affected breast, encompassing the entire tumor bed, the entire space posterior to the breast and the pectoral fascia, the interpectoral space (Rotter's lymph nodes), and any unswept low-mid axilla within the entire breast target volume.
3. Target Borders: • Superior border is the palpable/visible edge of the glandular tissue on CT. • Inferior border is the palpable/visible lower edge of the glandular tissue on CT. • Anterior border is 5 mm below the skin, but for small or thin breasts, it should be adjusted to 0.3 cm below the skin or even closer. • Posterior border is 1-2 mm behind the pectoral fascia (adjacent to the space below the breast), with no fat gap left and no inclusion of ribs/intercostal muscles. If regional lymph nodes have metastasized, include interpectoral lymph nodes and unswept axillary regions I and II. • Medial border is next to the sternum, extending at least to the inner edge of the peristernal vessels. • Lateral border is the palpable or CT-visible outer edge of the glandular tissue, anterior to the dorsal thoracic artery, anterior edge of the latissimus dorsi muscle.

2.2 Tumor Bed and CTV1:

1. Purpose: Suitable for patients who have undergone breast-conserving surgery. Boosting of the tumor bed is not mandatory and depends on the policy of each center. Boosting of the tumor bed is recommended under the following conditions: positive surgical margins, close margins (invasive cancer or DCIS with negative margins ≤2 mm), young age (<50 years).
2. Tumor Bed Definition: The area where the tumor and surrounding tissue have been excised, filled with serum exudate or plastic tissue.
3. Tumor Bed Borders: • Based on the location of the titanium clips. It is suggested that titanium clips be placed in five directions: left, right, up, down, and behind. • The extent of serum exudate, taking into account any within the glandular tissue and beneath the scar.
4. Tumor Bed CTV_1: • Includes the resected tumor bed extended by 10-15 mm into the glandular breast tissue and soft tissue. For patients undergoing segmental resection, this may be appropriately reduced, with a suggested 10 mm. If there's no glandular tissue outside the tumor bed, consider reducing it. In case of positive margins or presence of extensive intraductal component (EIC), severe atypical ductal hyperplasia (ADH), the range must be appropriately expanded.

2.3 Integrated Target Volume of the Entire Breast and Low-to-Mid Axillary Region (CTV_2):

1. Purpose: Suitable for patients who have undergone breast conservation surgery with sentinel lymph node biopsy.
2. Target Definition: The entire breast + lymph nodes in axillary regions I and II + interpectoral lymph nodes (Rotter's lymph nodes).
3. Target Borders: • For the entire breast, refer to Section1.1 Entire Breast Target Volume; Axillary Region I: anatomically marked by the small pectoral muscle, located on the lateral side of the muscle; Axillary Region II: follows the axillary vein, located posterior to the small pectoral muscle, and also includes the interpectoral space (Rotter's LN).

Boundary	Axillary Region I	Axillary Region II	Rotter's Lymph Nodes
Superior Border	Where axillary vessels cross the lateral edge of the small pectoral muscle	Where axillary vessels cross the medial edge of the small pectoral muscle	Including the cranial side of the axillary artery and 5 mm above the axillary vein
Inferior Border	Where the large pectoral muscle inserts into the rib	Where axillary vessels cross the lateral edge of the small pectoral muscle	Inferior border of axillary region II
Anterior Border	In front of the large pectoral and latissimus dorsi muscles	In front of the small pectoral muscle	Behind the large pectoral muscle

Posterior Border	In front of the subscapularis muscle	Ribs and intercostal muscles	In front of the small pectoral muscle
Medial Border	Outer edge of the small pectoral muscle	Inner edge of the small pectoral muscle	Inner edge of the small pectoral muscle
Lateral Border	Inner surface of the latissimus dorsi muscle	Outer edge of the small pectoral muscle	Outer edge of the small pectoral muscle

Supplementary **11** Dose distribution and organ endangerment limits

Target Volume	Dmax	Dmin
Whole Breast PTV_2	≤107%	≥90%
Tumor Bed PTV_1	≤107%	≥90%
Whole Breast and Low-to-Mid Axilla Integrated Target Volume PTV_2	≤107%	≥90%
Chest Wall PTV_CW	≤110%	≥90%
Supraclavicular (±intranodal clavicular) PTV_LN	≤110%	≥90%
Internal Mammary PTV_IMN	≤110%	≥80%

Supplementary 12. Organ dose/volume/impact data for routine split exposures (except where noted): QUANTEC

Organs	Volume	Type of irradiation (partial organ or specially indicated)	Observation index	Dose (Gy) or dose volume parameter	Incidence (%)	Dose volume parameter description
Spinal Cord	Partial spinal cord Thoracic medulla	3DCRT	Spinal cord lesions	Dmax=50	0.02	Includes all spinal cord cross-sections
Pharynx	pharyngeal constrictor muscle	3DCRT	Dysphagia and shortness of breath	Dmean＜50	＜20	
larynx	Total larynx	3DCRT	Edema	Dmean＜44	＜20	No chemotherapy, based on a single study of patients without laryngeal cancer
	Total larynx	3DCRT	Edema	V50＜27%	＜20	
lung	whole lung	3DCRT	Pneumonia	V20≤30%	＜20	Double lung. Slow dose response Without whole lung treatment irradiation
	whole lung	3DCRT	Pneumonia	Dmean=7	5	
	whole lung	3DCRT	Pneumonia	Dmean=13	10	
	whole lung	3DCRT	Pneumonia	Dmean=20	20	
	whole lung	3DCRT	Pneumonia	Dmean=24	30	
	whole lung	3DCRT	Pneumonia	Dmean=27	40	
Esophagus	Whole Esophagus	3DCRT	≥3 Grade acute esophagitis	Dmean＜34	5-20	Contains various dose limiting factors. Seems to be related to dose volume
	Whole Esophagus	3DCRT	≥ grade 2 acute esophagitis	V35＜50%	＜30	
	Whole Esophagus	3DCRT	≥ grade 2 acute esophagitis	V50＜40%	＜30	
heart	Pericardium	3DCRT	pericarditis	Dmean＜26	＜15	Based on individual studies
	Pericardium	3DCRT	pericarditis	V30＜46%	＜15	
	Whole heart	3DCRT	distant cardiac death	V25＜46%	＜1	High standards for assessing security based on predictive models
Liver	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜30-32	＜5	Exclude patients with existing liver disease or liver cancer
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜42	＜50	Patients with liver disease or hepatocellular carcinoma
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜28	＜5	
	Whole	3DCRT	Typical	Dmean＜	＜50	

	Liver - GTV		RILD	36		with a Child-Pugh rating of A, but not active hepatitis B, were included as observation indicators
	Whole stomach	3DCRT	Ulcer	D100＜45	＜7	

QUANTEC: Quantitative Analysis of Illumination Response in Clinically Normal Tissues; 3DCRT: Three-Dimensional Conformal Radiotherapy; GTV: Gross Tumor Volume; RILD: Radioactive Liver Injury; RTOG: Radiation Therapy Oncology Group of Amer

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RecurIndex-Guided postoperative radiotherapy with or without Avoidance of Irradiation of regional Nodes in 1-3 node-positive breast cancer (RIGAIN): a study protocol for a multicenter, open-label, randomized controlled prospective phase III trial

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RecurIndex-Guided postoperative radiotherapy with or without Avoidance of Irradiation of regional Nodes in 1-3 node-positive breast cancer (RIGAIN): a study protocol for a multicenter, open-label, randomized controlled prospective phase III trial

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ABSTRACT

Introduction

Postoperative radiotherapy in breast cancer patients with 1-3 lymph node metastases, particularly within the pT1-2N1M0 cohort with a low clinical risk of local-regional recurrence (LRR), has incited a discourse surrounding personalized treatment strategies. Multigene testing for Recurrence Index (RI) model capably differentiates patients based on their level of LRR risk. This research aims to validate whether a more aggressive treatment approach can enhance clinical outcomes in N1 patients who possess a clinically low risk of LRR, yet a high RecurIndex-determined risk of LRR. Specifically, this entails postoperative whole breast irradiation (WBI) combined with regional lymph node irradiation (RNI) following breast-conserving surgery (BCS) or chest wall irradiation (CWI) with RNI after mastectomy.

Methods and Analysis

The RIGAIN study is a multicenter, prospective, randomized, open-label, phase III clinical trial that is being conducted in China. In this study, patients with low clinical LRR risk but high RecurIndex LRR risk are randomly assigned in a 1:1 ratio to the experimental group or the control group. In the experimental group, RNI is performed and the control group omits RNI. Efficacy and safety analyses will be conducted, enrolling a total of 540 patients (270 per group). The primary endpoint is invasive disease-free survival, and secondary endpoints include any first recurrence, local-regional recurrence-free survival, distant metastasis-free survival, recurrence-free survival, overall survival, disease-free survival, breast cancer-specific mortality, and assessment of patient quality of life. The study began in April 2023 and with a follow-up period of 60 months after the last participant completes radiation therapy.

Ethics and dissemination

The study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-097-02, version 3.1). It adheres to the Helsinki Declaration and good clinical practice. Research findings will be submitted for publication in peer-reviewed journals.

Trial registration number NCT04069884

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The trial is designed as a multicenter, open -label, randomized, controlled phase III study.
- Introduced a multigene model to guide precision radiotherapy.
- This research utilizes IDFS as the primary endpoint.
- The trial is conducted only in one country (China).

INTRODUCTION

Patients with 1-3 axillary lymph node metastases constitute approximately 25 to 30% of early operable breast cancer cases. Radiotherapy plays a pivotal role in the comprehensive treatment of breast cancer[1,2]. However, the benefit of postoperative radiotherapy for N1 breast cancer patients,

particularly in terms of survival improvement, remains a topic of substantial debate. Studies conducted in the 1990s such as the Vancouver study, DBCG-82b/82c, and the early meta-analysis by EBCTCG (including the N1 subgroup) consistently demonstrated that postoperative radiotherapy significantly enhances disease-free survival (DFS) and overall survival (OS) for patients[3-7]. Consequently, N1 becomes a relative indication for postoperative radiotherapy. The 2011/2014 EBCTCG meta-analysis further suggested that postoperative radiotherapy could convert a 1.5% reduction in the 10-year any first recurrence rate (AFRR) into a 1% 20-year OS benefit[8,9]. The MA20 and EORTC 22922 studies published in 2015[10,11], which focused on T1-2N1 patients, especially those with high clinical risk of LRR and compared postoperative RNI after BCS or without RNI, found that more aggressive postoperative RNI for T1-2N1 patients could result in better distant metastases-free survival (DMFS), DFS, or breast cancer-specific mortality (BCSM). The Vancouver study's 20-year long-term follow-up results demonstrated the long-term OS benefit of postoperative radiotherapy in the N1 subgroup. These milestone studies further reinforced the value and recommendation of postoperative radiotherapy for N1 breast cancer patients, rendering N1 staging a strong relative indication for postoperative radiotherapy and increasing the number of patients actively accepting postoperative radiotherapy. Nevertheless, not all N1 patients will benefit from postoperative radiotherapy. Some real-world retrospective studies reveal limited LRR and/or survival improvement from postoperative radiotherapy, particularly RNI therapy, among certain N1 patients, especially those with relatively low clinical risk. Consequently, the necessity of radiotherapy for clinically low LRR risk N1 patients remains a topic of significant controversy and uncertainty. Clinical practice often presents varying professional recommendations for postoperative radiotherapy in low-risk N1 patients, resulting in the exclusion of a substantial number of patients solely based on traditional clinical and pathological features. However, this omission of RNI could lead to inadequate treatment, with potential implications for tumor recurrence, metastasis, and patient survival. Conversely, a uniform approach of postoperative radiotherapy for all clinically low LRR risk N1 patients would inevitably result in overtreatment and expose patients to additional risks such as radiation-induced injury and related complications, thereby impacting their quality of life[12-15].

When considering low-risk N1 breast cancer patients, then, the primary objective is to identify the actual high-risk patients concealed within the clinically low-risk population and to strategically administer postoperative radiotherapy. This represents one of the essential development directions of early breast cancer "precision radiotherapy" in the future. Achieving individualized and precise radiotherapy depends on the discovery of molecular genetic prediction models that can accurately predict local-regional recurrence risk in a scientific, reliable, and accessible manner. Currently approved multi-gene detection models abroad include Oncotype DX, MammaPrint, and EndoPredict. Oncotype DX is the most representative and extensively utilized multi-gene prognostic analysis method, primarily employed to guide early luminal low-risk patients to avoid adjuvant chemotherapy. Oncotype DX is currently more frequently used in the radiotherapy field to identify low-risk elderly N0 breast-conserving patients exempt from postoperative radiotherapy. Although the predictive value in the N1 population has initially demonstrated some clinical significance, contradictions exist between various research findings[16-19]. No prospective high-level evidence for multi-gene models predicting RNI benefits in N1 patients is currently available. The

clinical trial Tailor RT (MA 39) conducted by the Canadian Cancer Trials Group primarily investigates whether low-risk recurrence patients can be spared from postoperative radiotherapy or RNI. This is currently the only prospective, randomized, controlled phase III study internationally that utilizes a multi-gene predictive model to guide precise radiotherapy for N1 patients. The future research outcomes will primarily be applied to guide the omission of postoperative radiotherapy in clinically low LRR risk and genetically low-risk N1 patients. However, the significance of postoperative radiotherapy for patients with intersecting risks, especially those with clinically low-risk but genetically high-risk profiles, remains uncertain.

RecurIndex is the only risk prediction model developed based on the Chinese population for early-stage breast cancer. Consisting of 18 core genes and 10 IHC4 reference genes, it is capable of independently predicting the risk of LRR and distant metastasis[20-24]. Internal validation studies in Taiwan and external validation studies conducted in Singapore, Hong Kong, and the Fourth Affiliated Hospital of Hebei Medical University in China have all provided strong evidence of RecurIndex's predictive efficacy and its value in guiding radiotherapy for N1 patients[25,26]. Low-risk and high-risk patients identified by RI-LRR had 5-year LRR rates of 0% and 7%, respectively (P=0.0146). Compared to high-risk RI-LRR patients who did not receive postoperative radiotherapy, those who underwent postoperative radiotherapy demonstrated significantly improved rates of LRR and RFS, with percentages of 88.8% vs 74.1% (P=0.0071) and 79.4% vs 59.5% (P=0.0019), respectively. These results clearly indicate the significant benefits of postoperative radiotherapy in this patient population. To date, RecurIndex has become widely recognized and clinically implemented around the Asia-Pacific region. It has been incorporated into the "Expert Consensus on Multigene Testing for Adjuvant Therapy of Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Early Breast Cancer" in China and recommended in the "Chinese Society of Clinical Oncology (CSCO) Guidelines for the Diagnosis and Treatment of Breast Cancer 2022" for guiding precise postoperative radiotherapy in N1 patients. However, further high-level, randomized, controlled phase III clinical trials are needed to validate its clinical applications and expand its usage in the field. The most promising and crucial area for its application lies in guiding precise radiotherapy for N1 breast cancer patients.

In summary, we have begun conducting a multicenter, prospective, randomized, controlled phase III clinical study of individualized precision radiotherapy for clinically low LRR risk breast cancer patients with N1 guided by RecurIndex. This study aims to evaluate patients' local recurrence and distant metastasis risks, primarily investigating whether active postoperative radiotherapy can further improve clinical efficacy in N1 patients with clinically low risk but high RecurIndex LRR risk. The ultimate goal of this study is to provide high-level clinical evidence and reliable multi-gene recurrence risk prediction models to help achieve individualized precision radiotherapy for N1 breast cancer patients.

MATERIALS AND METHODS

The RIGAIN study is a multicenter, prospective, randomized, open-label, phase III clinical trial that is being conducted in China. The overall research process is illustrated in Figure 1. This study aims to screen postoperative patients with early-stage breast cancer (pT1-2N1M0) who have

completed standard systemic therapy and possess eligible pathological specimens for participation. The inclusion and exclusion criteria are listed in Table 1. RecurIndex testing will be performed using postoperative paraffin-embedded tissue sections from the primary lesion. The study is divided into a randomized controlled trial and an observational study based on clinical risk and RecurIndex LRR risk. Patients with low clinical risk but high RecurIndex LRR risk will be randomly assigned in a 1:1 ratio to either the experimental group (RNI) or the control group (No RNI), while patients with low clinical risk and low RecurIndex LRR risk will be included in the observational study. This article primarily focuses on the randomized controlled trial. The study participants will receive the following treatments. Experimental group: For patients who underwent BCS, RNI will be performed in combination with whole breast irradiation (WBI) + tumor bed boost irradiation. For patients who underwent mastectomy, chest wall irradiation (CWI) will be administered in combination with RNI. Control group: RNI will be omitted. For patients who underwent BCS, only WBI + tumor bed boost irradiation will be administered. For patients who underwent mastectomy, both RNI and CWI will be omitted. A comparative effectiveness analysis will be conducted. The study commenced in April 2023.

Randomization Method

Stratified randomization will be used for the randomized study. For the active postoperative radiotherapy trial involving the clinically low LRR risk but high RecurIndex LRR risk population, participants will be stratified by N1 status, surgical method, and enrolling hospital, and then randomized in a 1:1 ratio into the experimental and control groups.

Randomization stratification factors are as follows:

a) N1 status: N1mic or 1-2 LN macrometastasis (including N1sln), or 3 LN macrometastasis;

b) Surgical method: breast-conserving surgery or mastectomy; and

c) Multicenter enrolling hospital.

A central randomization system was developed by TaiMei Medical Technology Company to facilitate the randomization process. Statistical experts responsible for randomization designed the randomization parameters in advance, allowing the system to generate a random allocation table. The main clinical trial centers conduct eligibility screening for potential participants. Once deemed eligible, the researchers at each sub-center access the server via the internet and enter the information of the enrolled patients. The system then assigns a corresponding randomization number based on the random allocation table, determining the patient's placement in the respective study group.

Participants and recruitment

Patients will be recruited by radiation oncologists from each participating research center. For each interested patient, the clinician or clinical coordinator will provide a complete and comprehensive introduction to them or their designated representative, informing the patient about their rights, the risks involved, and the potential benefits they may receive to enhance their compliance with the protocol. Prior to enrollment, patients are required to sign an informed consent form, which will be kept in the Case Report Form (CRF). Patient registration is scheduled to begin on April 1, 2023, and is expected to continue for 5 years (tentatively until January 2028).

The final collection of data for the primary outcome measures is anticipated to be completed by December 2032.

Patient and public involvement

Neither patients nor the public were involved in the design, conduct, reporting, or dissemination plans of this study.

Objectives and endpoints

This study aims to evaluate whether adjuvant radiotherapy to the regional lymph nodes after breast-conserving surgery or chest wall plus regional lymph node radiotherapy after total mastectomy can further improve clinical outcomes in N1 patients with low clinical risk but high RecurIndex LRR risk.

The primary endpoint is invasive disease-free survival (IDFS). Secondary endpoints are any first recurrence (AFR), local-regional recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), recurrence-free survival (RFS), overall Survival (OS), disease-free survival (DFS), breast cancer-specific mortality (BCSM) and patient quality of life assessment. The specific definitions can be found in Table 2.

During the screening period and 3 months after the end of treatment, patients in each group fill out the quality of life questionnaire (EORTC QLQ-C30), as well as the breast cancer survival quality scale (EORTC QLQ-BS23) annually during the follow-up phase. (Supplementary 1 and 2).

RecurIndex Test

RecurIndex testing was performed using postoperative paraffin-embedded tissue sections from the primary lesion of the subjects. All sections were uniformly sent to the Jiangsu Simcere Pharmaceutical Co., Ltd., Jiangsu Simcere Diagnostics Co. for free testing, Ltd. Formalin-fixed, paraffin-embedded tissue blocks should be selected that cover the largest amount of tumor cells and meet the diagnostic criteria in appearance. Tissue sections with an excess amount of normal tissue, necrotic tissue, adipose tissue, or hemorrhagic tissue should not be sent for examination. The tumor cell content in the identified sections should be >50% for the test to be performed. A total of 10 consecutive sections are needed, each with a thickness of 5 microns. The sections can remain unstained and without coverslips. There is no need to oven-dry the sections; they can be air-dried naturally.

Safety Assessment Indicators

All patients participating in the RIGAIN study are required to undergo safety assessments, including acute radiation reactions and late radiation injuries for radiotherapy patients. The evaluation criteria and handling of injuries are detailed in Supplementary 3-7. Their treatment is shown in Supplementar 8. Including acute skin reactions to radiotherapy, symptomatic radiation pneumonitis, long-term cosmetic outcomes (BCS/reconstruction patients), skin fibrosis (total mastectomy patients), ischemic heart disease, upper limb edema[27,28], brachial plexus injury and second primary tumor[29].

Radiotherapy

General consideration

The overall treatment plan for each participant is determined by the researchers at the corresponding sub-center based on the participant's condition. Depending on their assigned group, patients will either undergo RNI or be exempted from it. Breast-conserving patients will all receive WBI. Patients should start radiotherapy within 8 weeks after completion of adjuvant chemotherapy. The regional lymph nodes include the supraclavicular lymph nodes and infraclavicular lymph nodes (unresected levels II/III axillary lymph nodes), with or without internal mammary lymph nodes (at least from the 1st to the 3rd intercostal space). For patients with minimally positive SLN and without ALND, the inclusion criteria encompass the low/intermediate axillary lymph nodes. The planned endocrine therapy and anti-HER2 treatment can be continued during the RT process.

Patient positioning and immobilization

The patient lies on a fixed device such as a breast support, vacuum bag, or foam pad. A CT scan is performed with a thickness of 3-5 mm, from the second cervical vertebra to the second lumbar vertebra. CT positioning includes surface marking, where lead wires are placed on the surgical scar of the primary lesion in breast-conserving patients or the chest wall scar in total mastectomy patients, as well as on the scar of the axillary sentinel/clearance lymph node incision. If there is a drainage site, it should also be separately marked with a lead wire or lead point.

Volumes of interest

The clinical target volume (CTV) and organs at risk (OAR) must be delineated on all CT slices, following the contouring guidelines of the Radiation Therapy Oncology Group (RTOG) and considering the actual situation at each CT slice. Detailed descriptions of CTV and OARs can be found in Supplementary 9. The margin between the planning target volume (PTV) and CTV depends on the institutional standards of each participating center, with a recommended minimum of 5 mm. Contours should be drawn according to the RTOG guidelines, including the ipsilateral and contralateral lungs, heart, humeral heads, and spinal cord.

External beam equipment and techniques

Radiation therapy techniques that can be employed include three-dimensional conformal radiotherapy (3DCRT), forward intensity-modulated radiotherapy (F-IMRT), inward intensity-modulated radiotherapy (I-IMRT), volumetric modulated arc therapy (VMAT), and helical tomotherapy (HT). Conventional radiotherapy (using a simulator for positioning and a 2D planning system to design treatment plans with external and tangential fields) and proton therapy techniques are not allowed. Some variations in treatment planning and implementation are permitted to accommodate the participating centers in adapting to the research protocol. However, it is strongly recommended that the treatment plans for enrolled patients at each center remain consistent to avoid confusion.

Dose prescription, fractionation

The whole breast target volume, or the integrated target volume of the whole breast and low to moderate axillary region, or the chest wall target volume, and the regional lymph node target volume receive a radiation dose of 5000 cGy in 25 fractions, delivered at a rate of 200 cGy per day, five days per week. Alternatively, a hypofractionated radiotherapy scheme can be chosen,

with a radiation dose of 4000-4256 cGy in 15 to 16 fractions. For breast-conserving patients, a sequential tumor bed boost is performed after completion of whole breast irradiation, as determined by individual center investigators. It can be delivered using conventional fractionation, with a dose of 1000 cGy in 5 fractions at a rate of 200 cGy per day, or by using hypofractionation, with a dose of 798 cGy-1064 cGy in 3 to 4 fractions at a rate of 266 cGy per day. If there are high-risk factors for local recurrence, such as positive surgical margins, close margins, or young age, the radiation dose for the tumor bed boost may be increased to 1400-1600 cGy in 7 to 8 fractions at a rate of 200 cGy per day.

DVH constraints

It is required that at least 95% of the prescribed dose to the PTV covers 95% of the PTV. The specific dose distribution is determined by each center's policy, with a recommended level as shown in Supplementary 10. For breast-conserving patients, it is recommended to achieve a central axis dose uniformity of ≤±7% for PTV_2 (whole breast or integrated target volume of whole breast and low to moderate axillary region) and PTV_1 (tumor bed), and to minimize the volume receiving ≥105% of the prescribed dose. The constraints for organs at risk should follow the Quantitative Analysis of Normal Tissue Effects in Clinical (QUANTEC) guidelines (see Supplementary 11).

Withdrawal from research and study termination

Termination of treatment

Research treatment will be terminated if any of the following conditions occur in the patient. The following are the criteria for the withdrawal or dropout of subjects:

1. The subject withdraws informed consent;
2. Any AE causes the subject to be unable to continue participating in the study;
3. The subject is lost to follow-up;
4. The subject does not comply with the study requirements and/or the investigator's instructions;
5. The subject has a concomitant illness or change in the subject's condition, and the investigator believes the subject is no longer suitable for the study treatment; or
6. For any other reason the investigator believes the subject is not suitable for continuing in the study.
7. If a subject drops out or withdraws, relevant safety and efficacy evaluations should be completed as soon as possible.

Study Termination

The trial will be terminated if any of the following situations occur during the trial:

1. Serious safety issues arise during the trial;
2. There is a major error in the study protocol;
3. The principal investigator voluntarily stops the trial; or
4. The administrative authority revokes the trial.
5. The termination of the trial may be temporary or permanent.

If the trial is terminated, all trial records should be retained for review.

Follow-up evaluation and toxicity assessment

The registration timeline, intervention measures, and assessments are presented in Supplementary 12. In the follow-up phase after radiotherapy, check-ups and assessments will be performed every six months until the occurrence of an endpoint event or the end of the study. For patients without postoperative radiotherapy, check-ups and assessments will be conducted every six months after the completion of adjuvant chemotherapy until the occurrence of an endpoint event or the end of the study. Effectiveness evaluations include tumor imaging examinations and assessments, brain MRI or CT, bone scans, quality of life questionnaires (EORTC QLQ-C30), and breast cancer-specific quality of life scales (EORTC QLQ-BS23). Safety evaluations include but are not limited to physical examinations, ECOG PS scores, pregnancy test checks, blood routine tests, blood biochemistry tests, adverse events, and serious adverse events. At the end of the study, participants will undergo physical examinations, performance status assessments (ECOG PS), blood routine tests, blood biochemistry tests, tumor markers, breast ultrasound/MRI, mammography, chest X-ray/CT, abdominal ultrasound/CT, and EORTC QLQ-C30 and EORTC QLQ-BS23 scoring. Evaluations of concomitant medications and adverse events are also required. The end-of-study visit window is 60 months after the last participant completes radiotherapy. For participants who are withdrawn or drop out before the end of the study, safety and effectiveness assessments will be conducted according to the requirements of end-of-study safety and effectiveness visits.

Data Management and Quality Assurance

In this study, electronic case report forms (eCRF) are used to collect data, and the EDC system designated by the principal investigator is used to complete the eCRF. Monitors verify the original data to ensure that the data entered into the eCRF by authorized trial center personnel (i.e., original data) is accurate, complete, and derived from original documents. Researchers and trial institutions must provide monitors with direct access to applicable source documents and reports for inspection and IEB/EC review. A Data Safety Committee has also been established, consisting of 5 members who are independent of the project team and have signed a research confidentiality agreement. The main tasks of the committee are to review and analyze positive results (recurrence and metastasis of subjects) and to understand the actual research results (without statistical analysis) when half of the subjects are enrolled. The committee will vote on whether it is necessary to adjust the research plan. This protocol does not include investigational drugs, and any toxicities that occur during treatment should be reported to the principal investigator and their ethics committee. In addition, the sub-centers should also report to the ethics committee of their institution. All serious adverse events and other adverse events must be recorded in the case report form.

Sample Size Estimation

This study is designed for superiority, referring to authoritative postoperative radiotherapy studies MA20[10] and EROTC22922[11] for N1 patients, in which the 5-year IDFS in the radiotherapy group and the control group were 90.7% vs 81.9% and 87.7% vs 77.1%, respectively. In the domestic RecurIndex external validation retrospective study[21] for N1 breast cancer patients, the 5-year IDFS in the high RI-LRR risk group was 81.1% vs 69.7% in the postoperative radiotherapy group and the control group, respectively. It is expected that the 5-year IDFS for the clinically low LRR risk and high RecurIndex LRR risk population in the experimental group and control group in this study will be 89% and 82%, respectively. The superiority margin is set to improve the primary

endpoint IDFS by ≥7% (HR=0.587) in the postoperative radiotherapy research group compared to the control group. With a one-sided significance level (α) of 0.025 and a power (1-β) of 0.8, assuming the experimental group performs better than the control group, the required sample size for each group was calculated as 216 cases per group using PASS15.0 software. The allocation ratio between the experimental and control groups was set at 1:1. Considering a 5-year enrollment period, 5-year follow-up period, and potential 20% dropout rate (mainly considering the need for further 10-year and 15-year long-term efficacy follow-up after reaching the 5-year endpoint), each group will need 270 cases, totaling 540 cases.

Statistical analysis

Descriptive statistical analyses will be conducted based on demographic characteristics such as age, gender, height, weight, and other baseline characteristics such as medical history. The Cox proportional-hazards model will be used for analysis of the primary endpoint. The Hazards Ratio and its 95% confidence interval will be calculated, including stratification factors and other covariates. Additionally, the Cox proportional-hazards model without covariates will be used to support the analysis results of the primary endpoint. Moreover, the Kaplan-Meier (KM) method will be used to calculate the median invasive disease-free survival for the two groups (experimental group vs control group), including 95% confidence intervals. KM plots will be used to illustrate the time trends of IDFS. For the secondary endpoints, the AFR of each group will be calculated, as well as the corresponding 95% Clopper-Pearson confidence intervals. LRFS, DMFS, RFS, OS, DFS, and BCSM will be analyzed for median values using the KM method (including 95% confidence intervals). The overall changes in EORTC QLQ-C30 and EORTC QLQ-BS23 scores from baseline will be summarized. Safety analysis will be conducted by summarizing adverse events, changes in laboratory test results, changes in vital signs, and study treatment exposure. The results will be reported by treatment group. All adverse events during treatment, grade 3 or higher TEAEs, serious adverse events (SAEs), radiotherapy-related SAEs, and TEAEs leading to study termination will be summarized by organ system, preferred term, and group in terms of numbers and percentages. A p value ≤0.05 in a two-tailed test will be considered statistically significant. Statistical analyses will be performed using SPSS V.25.0 (Statistical Package for Social Sciences) and STATA V.14.

Ethics and dissemination

This study has obtained approval from the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-097-02, version 3.1), as well as approval from the respective participating centers' ethics committees. The study is being conducted in accordance with the Helsinki Declaration and good clinical practice. Approval from the Chinese Human Genetic Resources Office was obtained on January 6, 2021, with the reference number 2020SQCJ2358. The study was registered on ClinicalTrials.gov on July 7, 2022, with the registration number NCT04069884. The research findings will be published in peer-reviewed journals. The authors will be individuals who have made significant contributions to the study, design, and implementation. Any modifications to the study protocol and informed consent documents must be reviewed and approved by the Ethics Committee before implementation.

Confidentiality and protection of participants' rights and interests

Researchers are required to explain to participants that participation in the clinical trial is voluntary, and that they have the right to withdraw from the study at any stage without affecting their medical treatment and rights. Personal information of participants will be kept confidential. Participants should be informed about the nature, purpose, potential benefits, and possible risks of the clinical trial, as well as alternative treatment options. Researchers should ensure that the rights and obligations of participants, as stipulated in the declaration, are protected. Participants should be given adequate time to consider whether to participate and to sign the informed consent form.

Discussion

The RIGAIN study is a multicenter, open-label, randomized controlled phase III clinical trial. Our objective is to precisely assess patients with clinically low LRR risk N1 breast cancer who, if identified as high-risk for LRR by the RecurIndex test, may receive enhanced clinical efficacy from active RNI after BCS or CWI with RNI after mastectomy. The aim is to accurately identify patients who would benefit from intensified radiotherapy. In addition, we have established an observational study to investigate the potential to exclude RNI for patients who are clinically low LRR risk and are identified as low risk for LRR by the RecurIndex. The primary endpoint of the study is LRR, with the aim of identifying truly low-risk patients for whom radiotherapy can be safely omitted from planned treatment regimens. Similarly, the MA39 study defines a clinically and genetically low-risk LRR N1 population (age ≥40 years, luminal A type, Oncotype DX score <18) based on comprehensive clinical pathology, molecular subtype, and a multigene model. The anticipated results from the MA39 study could potentially guide personalized RNI decisions for patients who are found to be both clinically and genetically low risk. However, this study also has limitations. Firstly, the multi-gene models used in this study were developed to predict the risk of distant metastasis, and there may be inconsistencies between the occurrence of LRR and the risk of distant metastasis in clinical patients. Secondly, future research results are primarily intended to guide the omission of postoperative radiotherapy in clinically low-risk and genomically low-risk N1 patients. However, the significance of postoperative radiotherapy in patients with intersecting risks, particularly those who are clinically low-risk but genomically high-risk, remains unclear. Lastly, this study does not provide direct evidence for the application of Oncotype DX in guiding treatment decisions for Asian patients.

Traditionally, postoperative radiotherapy has been lauded for decreasing LRR and for helping to diminish the risk of distant metastases[10,11]. This has lead to long-term improvements in DFS and BCSS, providing the ultimate benefit to patients. Notably, this is partly attributed to the prevention of reseeding from recurrences. Another part is attributed to the radiation-induced abscopal killing effect (RIAKE), which refers to a series of immunological responses induced by local high-dose radiotherapy that culminate in the elimination of tumors distant from the irradiation site[27]. In the context of postoperative radiotherapy for patients with regionally lymph node-positive breast cancer, the abscopal effect is most pronounced in patients classified as pN1, where the survival benefit is most conspicuous[9]. Compared to mastectomy, BCS better preserves the immune microenvironment, thereby enhancing the transformation and activation of the immune response following postoperative radiotherapy. This is the primary reason for our selection of IDFS as the main endpoint in this study.

Oncotype DX and MammaPrint assays primarily assess the overall recurrence risk and mainly guide chemotherapy and endocrine treatment[16,18,30]. Previous studies have indicated a high concordance in predicting the risk of distant metastases between the RecurIndex and the MammaPrint and Oncotype DX assays. However, some discrepancies exist in assessing the risk of LRR. The TAILORx study indicated that the RecurIndex predictive model may identify patients at risk of locoregional recurrence more accurately than the Oncotype DX[16,31,32].

The RecurIndex predictive model stands out amongst various multigene prediction models in early-stage breast cancer with the following unique characteristics and advantages: 1. Unlike other models that only assess overall recurrence risk and are more biased towards the risk of distant metastases, RecurIndex can independently assess both the risk of locoregional recurrence and distant metastases, making it more suitable to guide precision radiotherapy; 2. RecurIndex demonstrates predictive efficacy in populations with HER2 overexpression and triple-negative breast cancer, potentially serving as a precise predictor of locoregional recurrence risk in patients with these two types of N1mic tumors, which could help guide individualized radiotherapy decisions.

The study focuses on the RecurIndex risk prediction model with the aim of guiding postoperative individualized radiotherapy for pT1-2N1M0 breast cancer patients. Particular attention is paid to the "clinically low LRR risk" but "genetically high-risk" population to explore and validate the clinical benefits of postoperative radiotherapy. The study design stands out for its clinical applicability and innovation as well as strict adherence to ethical and clinical practice standards. It effectively addresses the research gap in precise radiotherapy for N1 patients with overlapping risk profiles, both domestically and internationally. The study could potentially revolutionize the practice of postoperative radiotherapy by transitioning from a discretionary approach solely based on clinical and pathological information to an individualized optimization guided by clinical-genetic risk.

We anticipate that the RIGAIN study will generate high-quality evidence, establishing a precise risk assessment framework to guide optimized radiotherapy decisions for N1 breast cancer patients.

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Contributors

XH, YT and ZB designed the original protocol for the study. JC contributed to study management. JL, XH, YT and ZB drafted the manuscript. JL submitted the study. YT and ZB performed the sample size calculation and data analysis. RD and FW offer genetic testing. XH, JL, YT, JC, SH, AZ, LZ, YW, ZL, HY, XX, JC, XW-L, XL, XZ, WZ and XY participated in enrollment, treatment and follow-up of patients.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

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Table1. Eligibility criteria for the study

Inclusion criteria 1. Age ≥18 years, ≤70 years; 2. ECOG PS ≤2 (Supplementary 13); 3. Postoperative pathology confirms the diagnosis of invasive breast cancer; 4. Meets the clinical definition of low risk: ① Axillary lymph node micrometastasis (N1mic), or ② N1 patients who meet all of the following conditions: a) Age ≥40 years; b) Lymphovascular invasion (LVI) negative or limited to individual or small foci of LVI (excluding extensive or large amounts of LVI); c) Three clinical molecular subtypes (Luminal A type, Luminal B1 type, and Luminal B2 type) are allowed in this study: ER-positive (ER≥1%) and HER2-negative, or ER-positive (ER≥1%) and HER2 overexpressing, respectively. 5. Postoperative pathological diagnosis of axillary lymph node status as any of the following: a. Sentinel lymph node biopsy or axillary lymph node dissection with micrometastasis (N1mic), b. Sentinel lymph node biopsy with 1-2 lymph node macrometastasis (N1sln), c. Sentinel lymph node biopsy + axillary lymph node dissection or simple axillary lymph node dissection with 1-3 lymph node metastasis (N1); 6. The primary tumor and breast underwent breast-conserving surgery or mastectomy ± breast reconstruction (autologous/prosthetic); 7. A thorough systemic examination (e.g., chest X-ray, ultrasound, CT, etc.) within 3 months before randomization for radiotherapy must confirm no distant metastasis; 8. Mammography and/or MRI within 12 months before surgery or randomization for radiotherapy must confirm no contralateral breast cancer; 9. Postoperative completion of at least 4 cycles of adjuvant chemotherapy containing anthracycline or taxane regimens; 10. Radiotherapy must be performed sequentially after the completion of all adjuvant chemotherapy, starting no later than 8 weeks after the end of chemotherapy; 11. Patients must have sufficient postoperative paraffin tissue sections of the primary tumor for RecurIndex testing; 12. No history of other malignant tumors, except for basal cell carcinoma of the skin; and 13. Signed informed consent before the start of the study.
Exclusion criteria 1. Confirmed T3-4, N0, N2-3, M1 stage disease before postoperative radiotherapy enrollment; 2. Received any neoadjuvant treatment before surgery, including chemotherapy, endocrine therapy, targeted therapy, or radiotherapy; 3. Patients who underwent mastectomy and only had sentinel lymph node biopsy; 4. History of contralateral breast cancer or other second primary malignant tumor (excluding basal cell carcinoma of the skin and cervical carcinoma in situ); 5. Previous history of chest radiotherapy; 6. Presence of severe heart, lung, liver, kidney, hematopoietic system, or nervous system diseases, or mental disorders; 7. Presence of scleroderma or active systemic lupus erythematosus or other autoimmune diseases; 8. Pregnant and breastfeeding patients.

Table 2. The specific definitions of the study endpoints

IDFS	The time from the day the subject is randomized to the earliest occurrence of invasive cancer local recurrence, distant metastasis, or death, but does not include contralateral breast second primary cancer.
AFR	Any ipsilateral chest wall, breast, regional lymph node recurrence, or distant metastasis event that occurs during the follow-up period.
LRFS	The time from the day the subject is randomized to the earliest occurrence of ipsilateral chest wall, breast, or regional lymph node recurrence or death.
DMFS	The time from the day the subject is randomized to the earliest occurrence of distant metastasis or death.
RFS	The time from the day the subject is randomized to the earliest occurrence of ipsilateral chest wall, breast, regional lymph node recurrence, distant metastasis, or death.
OS	The time from the day the subject is randomized until the patient's death.
DFS	The time from the day the subject is randomized to the recurrence of the disease or the patient's death due to disease progression.
BCSM	The time from the day the subject is randomized to death from breast cancer.

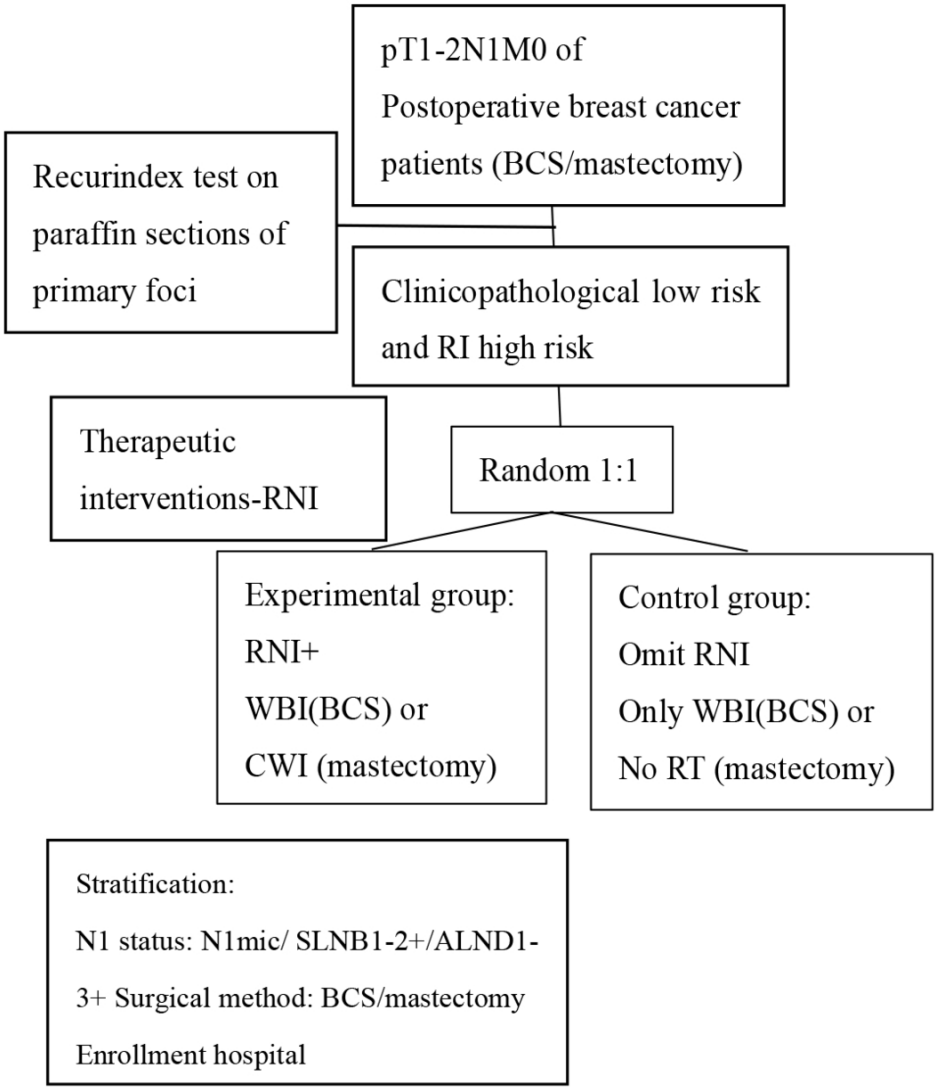

Figure 1 Research Process
Abbreviation: BCS, Breast conservative surgery; RI, Recurrence Index; RNI, Regional lymph node irradiation; WBI, Whole breast irradiation; CWI, Chest wall irradiation; RT, Radiotherapy; SLNB, Sentinel lymph node biopsy; ALND, Axillary lymph node dissection

90x114mm (300 x 300 DPI)

Spplementary 1. Quality of Life Questionnaire EORTC QLQ-C30 (version 3)

We are interested in learning some information about you and your health status. Please answer all of the following questions independently and circle the answer that is most appropriate for you. There are no "correct" or "incorrect" answers. The information you provide will be kept strictly confidential.

Please fill in your last name: _____

Date of birth (year, month, day): _____

Today's date (year, month, day): _____

	No	A little	Some	Very much
1.Do you feel difficulty when you do some laborious movements, such as lifting heavy shopping bags or luggage?	1	2	3	4
2. Do you find it difficult to walk long distances?	1	2	3	4
3. Do you find it difficult to walk short distances outdoors?	1	2	3	4
4. During the day, do you have to lie in bed or sit in a chair?	1	2	3	4
5. Do you need assistance with eating, dressing, washing or going to the bathroom?	1	2	3	4
In the past week:	1	2	3	4
6. Are your work or daily activities limited by physical ability?	1	2	3	4
7. Are your hobbies and leisure activities physically limited?	1	2	3	4
8. Do you ever feel short of breath?	1	2	3	4
9. Have you ever had any pain?	1	2	3	4
10. Have you ever needed rest?	1	2	3	4
11. Have you ever felt sleep deprived?	1	2	3	4
12. Have you ever felt weak?	1	2	3	4
13. Have you ever felt a lack of appetite?	1	2	3	4
14. Have you ever felt nauseous and wanted to vomit?	1	2	3	4
15. Have you ever vomited?	1	2	3	4
16. Have you ever had constipation?	1	2	3	4
17. Have you ever had diarrhea?	1	2	3	4
18. Do you ever feel tired?	1	2	3	4
19. Does pain interfere with your daily activities?	1	2	3	4
20. Do you have difficulty concentrating on things, such as reading the newspaper or watching TV?	1	2	3	4
21. Do you ever feel nervous?	1	2	3	4
22. Do you ever feel worried?	1	2	3	4
23. Do you ever feel easily irritated?	1	2	3	4
24. Do you ever feel depressed?	1	2	3	4
25. Do you ever have trouble remembering things?	1	2	3	4
26. Has your medical condition or treatment process interfered with your family life?	1	2	3	4
27. Has your medical condition or treatment interfered with your social activities?	1	2	3	4
28. Has your medical condition or treatment process caused you financial difficulties?	1	2	3	4

For the following questions, the numbers 1-7 represent a scale from "very poor" to "very good".

29. How would you rate your overall health in the past week?

1	2	3	4	5	6	7
very poor" to						very good

30. How would you rate the overall quality of your life in the past week?

1	2	3	4	5	6	7
very poor" to						very good

Patients sometimes have the following clinical symptoms. Please indicate the extent of these clinical symptoms or problems you have had in the past week, circling the answer that best applies to you.

	No	A little	Some	Very much
31. Do you cough a lot?	1	2	3	4
32. Do you cough up blood (blood in sputum)?	1	2	3	4
33. Do you feel short of breath when you rest?	1	2	3	4
34. Do you feel short of breath when you take a walk?	1	2	3	4
35. Do you feel short of breath when climbing stairs?	1	2	3	4
36. Have you ever had pain in your mouth or tongue?	1	2	3	4
37. Have you ever had difficulty swallowing?	1	2	3	4
38. Have you ever had tingling/numbness in your hands and feet?	1	2	3	4
39. Have you ever had hair loss?	1	2	3	4
40. Have you ever had chest pains?	1	2	3	4
41. Have you ever had pain in your arms or shoulders?	1	2	3	4
42. Have you ever had any pain in other parts of your body?	1	2	3	4
If yes, please write down the area:				
43. Have you ever taken any painkillers?				
1.Yes 2.No				
If you have used it, does it help much with pain?	1	2	3	4

Supplementary 2. Breast Cancer Survival Quality Scale EORTC QLQ-BR23

Please recall if you have experienced any of these symptoms or the extent of the problem and tick the appropriate number"√"

In the past 1 week	No	A little	More	A lot
1. Do you have dry mouth?	1	2	3	4
2. Do your food and drinks taste different than usual?	1	2	3	4
3. Do your eyes hurt, feel uncomfortable, or tear up?	1	2	3	4
4. Do you have hair loss?	1	2	3	4
5. If you have hair loss, does it bother you?	1	2	3	4
6. Do you feel sick or uncomfortable?	1	2	3	4
7. Is your face red and hot?	1	2	3	4
8. Do you have a headache?	1	2	3	4
9. Do you feel less physically attractive due to illness or treatment?	1	2	3	4
10. Do you feel less attractive as a woman due to illness or treatment?	1	2	3	4
11. Do you have difficulty looking at your naked body?	1	2	3	4
12. Are you dissatisfied with your body?	1	2	3	4
13. Are you worried about your future health?	1	2	3	4

In the past 4 week				
14. How interested are you in sex?	1	2	3	4
15. How active are you sexually (do you have sex often)? (With or without sex?)	1	2	3	4
16. If you have sex, to what extent does it bring you pleasure?	1	2	3	4

In the past 1 week
17. Do you have pain in your arm or shoulder?
18. Is your arm or hand swollen?
19. Do you have difficulty lifting or moving your arm to the side?
20. Do you have pain in the area of your affected breast?
21. Is the area of your affected breast swollen?
22. Do you have hypersensitivity in the affected breast area?
23.Do you have skin problems (e.g. itching, dryness, flaking) in the affected breast area?

Supplementary 3. Evaluation criteria for common adverse events (CTCAE Version 4.03)

(excerpt, normal common adverse event evaluation criteria is grade 0)

Adverse Events	Grading				
	1	2	3	4	5
Hemoglobin g/L	Normal value -10.0	10.0-8.0	8.0-6.5	<6.5	
Leukocytes(10^9/L)	Normal value-3.0	3.0-2.0	2.0-1.0	<1.0	
Neutrophils(10^9/L)	Normal value-1.5	1.5-1.0	1.0-0.5	<0.5	
Platelets(10^9/L)	Normal value-75	75-50	50-25	<25	
Transaminase ALT/AST	≤2.5×N	2.6-5.0×N	5.1-20×N	>20×N	
Alkaline phosphatase	≤2.5×N	2.6-5.0×N	5.1-20×N	>20×N	
Bilirubin	ULN-1.5×N	1.5-3.0×N	3.0-10×N	>10×N	
Creatinine Cr	ULN-1.5×N	1.5-3.0×N	3.0-10×N	>10×N	
Weight gain/loss	5.0-10%	10-20%	≥20%		
Vomiting	Vomiting 1 time in 24h during treatment	Vomiting 2-5 times in 24h during treatment	Vomiting ≥ 6 times in 24h during treatment or requiring fluids	Life-threatening and requires urgent treatment	Death
Coughing sputum	Occasional/mild coughing of sputum	Moderate cough and sputum; interferes with instrumental daily life	Persistent heavy coughing and limited personal self-care		
Pneumonia	Asymptomatic; clinical examination or diagnostic findings only; no intervention required	Symptomatic (mild cough and/or dyspnea, with or without fever); requires clinical intervention; interferes with instrumental daily	Severe symptoms; limited personal autonomy; need for oxygen	Life-threatening respiratory dysfunction; requiring urgent treatment (tracheotomy or intubation)	Death

		life			
Acute coronary syndrome		Symptomatic, progressive angina; normal cardiac enzymes; hemodynamically stable	Symptomatic, unstable angina with/ or acute myocardial infarction, abnormal cardiac enzymatic parameters, hemodynamically stable	Symptomatic, unstable angina with/ or acute myocardial infarction, abnormal cardiac enzymatic parameters, hemodynamic instability	Death
Left ventricular systolic insufficiency			Symptoms of decreased ejection fraction	Uncontrollable heart failure with declining ejection fraction requiring urgent intervention	Death
Heart Failure	Asymptomatic, with abnormalities detected by laboratory tests (e.g., natriuretic peptide) or cardiac imaging	Mild to moderate symptoms with activity or exercise	Symptoms occur at rest or with light activity or exercise; requires treatment	Life-threatening; requires urgent treatment (e.g. continuous infusion therapy or mechanically assisted circulation)	Death
Limb edema	Comparison using the greatest difference in volume or circumference, with 5% to 10% variation between limbs; edema or blurred anatomy that can only be detected on close examination	Comparison using the largest difference in volume or circumference, 10% <~30% difference between limbs; disappearance of skin folds; apparent loss of limb anatomy, change in	>30% volume variation between limbs; severe changes in limb shape; limited personal autonomy		

		shape; interferes with instrumental daily living			
Neurotoxicity - Sensory	Mild sensory abnormalities (including paresthesia), absence of deep tendon reflexes	Moderate objective sensory deficit or sensory abnormalities (including tingling)	Severe objective sensory loss or sensory abnormalities that affect daily life	Persistent sensory loss, affecting function	Death
Neurotoxicity-motor	Self-perceived weakness with no objective findings	Moderate self-conscious weakness; no significant functional impairment	Self-perceived weakness with functional impairment	Paralysis	Death

ULN, Upper limit of normal value

Supplementary 4. Scoring criteria for acute radiation reactions (RTOG/EORTC 1995)

Organ Tissue	Grading				
	0	1	2	3	4
Skin	No change	Punctate skin erythema, alopecia, dry peeling or decreased sweating	Marked erythema, patchy wet peeling or moderate edema of the skin	Presence of fused desquamation or s edema	Skin ulcers, bleeding or necrosis
Larynx	No change	Mild to moderate hoarseness/cough without cough suppressants/mucosal edema	Persistent hoarseness but vocalization/involved otalgia, sore throat, flaky	soft speech, sore th or involved otalgia requiring	Significant dyspnea, wheezing, hemoptysis requiring tracheotomy or intubation
Pharynx and esophagus	No change	Mild dysphagia requiring general analgesia or/non-narcotic analgesia/need for semi-liquid diet	Moderate dysphagia/narcotic analgesia/fluid	narcotics / fused fibrin exudate, marked arytenoid edem	Complete obstruction, ulceration ulceration, perforation, sinus tract
Lungs	No change	Mild symptoms, mild dry cough or exertional dyspnea, may be associated with imaging changes	Moderate symptoms, persistent cough requiring narcotic cough suppressant treatment or dyspnea with mild activity	Severe dysphagia dehydration or weight loss >15% need for gastric feeding or intravenous fluids Severe cough or dyspnea at rest with severe symptoms, ineffective sedation or cough suppressants, clinical and imaging evidence of acute pneumonia, requiring intermittent oxygen or hormonal therapy	Severe respiratory insufficiency requiring continuous oxygen or assisted ventilation
Heart	No change	Asymptomatic but objective evidence of ECG changes or	Symptoms not requiring specific treatment with ECG	Congestive heart failure, angina pectoris or pericardial disease for	Congestive heart failure, angina pectoris,

		pericardial abnormalities, no evidence of other cardiac disease	changes and imaging changes of congestive heart failure or pericardial disease	which drug therapy is effective	pericardial disease or arrhythmias that have not responded to non-surgical treatment

Supplementary 5. Scoring criteria for late radiation injury (RTOG/EORTC 1995)

Organ Tissue	Grading				
	0	1	2	3	4
Skin	No change	Mild atrophy, hyperpigmentation, partial hair loss	Lamellar atrophy, moderate capillary dilatation, total hair loss	Significa ophy, marked c ary dilation	Ulcers
Subcutaneous tissue	No change	Mild sclerosis (fibrosis) and loss of subcutaneous adipose tissue	Moderate fibrosis but asymptomatic, slight constriction of irradiated field <10% of the side length	Severe s is and loss of subcu us tissue. Constric of the irradiated >10% border le g	Necrosis
Lungs	No change	Asymptomatic or mildly symptomatic (dry cough), mild imaging signs	Moderate symptomatic pulmonary fibrosis or pneumonia (severe cough), hypothermia, patchy imaging	Severe s mptomatic pulmonary fibrosis or pneumonia with dense imaging changes	severe respiratory insufficiency requiring continuous oxygenation or assisted ventilation
Heart	No change	Asymptomatic or mildly symptomatic; temporary T-wave inversion and ST changes; sinus tachycardia >110 beats/min at rest	Moderate exertional angina; mild pericarditis; normal heart size; persistent T-wave abnormalities and ST changes; low QRS waves	Severe angina pectoris; pericardial effusion; constrictive pericarditis; moderate heart failure; cardiac enlargement; abnormal electrocardiogram	pericardial tamponade; severe heart failure; severe constrictive pericarditis

Supplementary 6. Harris Cosmetic Grade Rating of Cosmetic Breast Preservation/Reconstructive Surgery Results

Level	Double breast symmetry	Double nipple level gap	Breast shape on the affected side	Skin
Excellent, Good	Symmetries	≤2cm	No significant difference with the healthy side, normal appearance, no deformation of the breast lift due to scarring, no difference between the affected side and the healthy side in feel	Normal
General	Symmetries	2cm-3cm	The shape of the affected breast is basically normal or slightly smaller than the healthy side, and the feel of the affected side is slightly worse.	Lightened or shiny color
Bad	Obvious asymmetry	＞3cm	The appearance of the affected side of the breast changes and is significantly smaller than the healthy side, and feels poorly in the hand	Thick, rubber-like, rough

Supplementary 7. Baker's classification of the prosthetic envelope

Grading	Breast Implants
I (no accessible envelope)	Breast implants feel as soft as non-operated breasts
II(Lightly hardened)	The softness of the breast is slightly worse, the implant can be touched but not seen
III(Heavy hardening)	Harder breasts, implants can be easily touched out or visible deformation of the implant
IV(severe contracture)	Breasts are hard, painful when touched, skin temperature becomes cold, deformation is obvious

Only Baker grade III and IV are defined as periosteal contracture and require reoperation

Supplementary **8.** **Radiotherapy-related adverse reactions and their treatment**

1 Radiation-induced skin damage

Early skin reactions are those that occur within three months after the start of radiotherapy and are the most common complications in breast radiotherapy. Approximately 92% of patients receiving post-lumpectomy radiotherapy will experience acute radiation-induced skin reactions, mostly grade 1 or 2 mild reactions, with a wet desquamation incidence rate of about 3%. Patients undergoing mastectomy will almost always experience acute radiation-induced skin reactions, mostly grade 2 reactions, with a wet desquamation incidence rate of about 10-20%.

Prevention is the main approach to managing radiation-induced skin complications. For grade 1 and 2 injuries, conservative treatment is primarily used. Patients should wear loose, cotton open-front underwear, avoid friction and pressure on the skin in the irradiation area, avoid using irritating products such as soap and shower gel, avoid bathing with hot water or showering the irradiation area, and not apply chemical ointments or adhesive tape. If the skin is red, swollen, itchy, or painful, do not scratch it or apply medication randomly. Follow the doctor's advice for medication, such as triethanolamine cream, compound vitamin B12 solution, and medical radiation protectants. Wet dermatitis can be treated with exposure therapy, keeping the area dry and avoiding secondary infections. Wet dermatitis that does not heal after more than two months may develop into skin necrosis, often requiring surgical treatment, with skin grafting for larger areas.

Late skin reactions include local hyperpigmentation, telangiectasia, atrophy, and fibrosis. For chronic radiation dermatitis with recurrent ulceration and significant worsening, surgery is often used to prevent malignant transformation.

2 Pharyngeal and esophageal reactions

Irradiation of the supraclavicular area can cause pharyngeal pain and difficulty swallowing, which are generally mild and self-limiting. Prevention methods include using new radiotherapy techniques, accurate positioning, precise delineation of the target area, rational design of radiation fields, and reducing or avoiding irradiation of organs at risk. Symptomatic support treatment is provided for severe reactions.

3 Radiation-induced lung injury

Radiation-induced lung injury includes early radiation pneumonitis occurring within 3 months after radiotherapy and late radiation-induced pulmonary fibrosis occurring after 3 months. Approximately 2/3 of patients will develop asymptomatic radiation pneumonitis, which does not require treatment. The incidence of symptomatic radiation pneumonitis is between 1% and 5%, usually occurring within 2 months after radiotherapy or within 6 months after radiotherapy. Patients have symptoms and signs of pneumonia, which can manifest as cough, sputum, or fever, and in severe cases, dyspnea and hypoxia. In particular, when imaging examinations (chest X-rays and CT scans) show inflammatory exudative changes in the lung tissue within the irradiation field, symptomatic radiation pneumonitis can be diagnosed after excluding lung metastasis and tuberculosis. Supportive treatment, including hormones, oxygen therapy, and even mechanical ventilation, can provide complete relief, but some patients may still develop pulmonary fibrosis within 6-12 months, even with treatment. Pulmonary fibrosis is a late injury caused by damage to the lung interstitium and pleura, and in severe cases, it can be life-threatening.

There is currently no specific treatment for radiation pneumonitis, so prevention is more important than treatment. For patients undergoing whole-breast irradiation alone, it is recommended to use a dose-volume constraint of V20 < 22% for the ipsilateral lung. For those receiving irradiation of the supraclavicular lymph node region, a dose-volume constraint of V20 < 34% and V30 < 22% should be used for the ipsilateral lung to further evaluate the overall radiotherapy plan.

4 Radiation-induced heart damage

Radiation-induced heart disease (RIHD) initially manifests as acute pericarditis and later as coronary artery disease, chronic pericarditis, myocardial fibrosis, cardiomyopathy, heart valve damage, and cardiac conduction abnormalities. A 2013 New England Journal article reported that for every 1 Gy increase in the average dose to the heart, the incidence of major coronary events increased by 7.4%.

Reducing the risk of RIHD is also focused on prevention. The most fundamental measure is to minimize or avoid radiation exposure to the heart during radiotherapy. The Chinese Anti-Cancer Association Breast Cancer Diagnosis and Treatment Guidelines and Standards (2015 Edition) recommend that the average radiation dose to the heart should be assessed to be at least below 8 Gy. It is recommended to limit the heart's V30 to less than 10%. In addition, for high-risk populations of RIHD or those with cardiovascular disease, drugs that have a protective effect on the cardiovascular system should be used as soon as possible. Regular cardiac ultrasound follow-ups should be conducted during the follow-up phase.

5 Upper limb edema

Edema in the affected upper limb is one of the common complications after breast cancer surgery and/or radiotherapy, and the extent of surgery is an important influencing factor. AMAROS research reported that the 1-year, 3-year, and 5-year lymphedema incidence rates for the ALND group were 28%, 23%, and 23%, respectively, significantly higher than the 15%, 14%, and 11% for the SLNB + axillary radiotherapy group. The incidence of upper limb lymphedema after axillary lymph node biopsy alone is 5%. Edema caused by radiotherapy usually occurs 1 to 2 months after the end of radiotherapy. Depending on the time of onset, upper limb edema can be divided into early edema and delayed edema occurring after several years. Upper limb edema caused by tumor recurrence in the axilla and supraclavicular region is not considered a true post-treatment complication.

The main prevention method for upper limb edema is to reduce axillary dissection, and postoperative progressive functional exercise is the key to preventing upper limb edema. When upper limb edema occurs, manual massage or compression therapy can be used.

6 Brachial plexus nerve injury

Radiation-induced brachial plexus nerve injury is a rare late complication after breast cancer radiotherapy, with an incidence rate of 1%-4%. Early symptoms include sensory and motor disorders in the affected limb and pain, often accompanied by severe nocturnal pain. Some cases may also have lymphedema, with progressively worsening functional impairment. In the late stage, this can lead to the loss of function of the entire limb, causing lifelong disability for the patient and severely affecting the patient's daily life and rest, with a significant impact on their mental health and quality of life. A preliminary diagnosis can be made based on the patient's radiotherapy history, asymptomatic intervals, and clinical features in clinical practice. However, it is necessary to rule out brachial plexus nerve injury caused by tumor metastasis or compression.

Radiation-induced brachial plexus nerve injury is irreversible, and there is currently no ideal treatment

method, so prevention is crucial. It is essential to strictly follow the indications for radiotherapy in the lymphatic drainage area and pay attention to the radiotherapy range and radiation dose. For cases without severe pain, active measures should be taken to improve the blood supply of the nerves and surrounding soft tissues, and the earlier the diagnosis and treatment, the better the results. For advanced cases, treatment is aimed at relieving pain and improving quality of life.

7 Second primary tumors

Second primary tumors that can occur after breast cancer treatment include contralateral breast cancer and other malignant tumors such as lung cancer and soft tissue sarcomas. If these second primary tumors can be diagnosed and treated early, they do not affect the patient's survival. Therefore, regular follow-up of patients should be strengthened in clinical practice.

8 Rib fractures

The incidence is less than 1%. In most cases, patients have no noticeable symptoms, and fractures are discovered during bone scans or X-ray examinations. A small number of patients may experience chest wall or rib pain, which generally heals on its own without the need for special treatment.

9 Other side effects

During radiotherapy, patients may experience mild loss of appetite and fatigue. Therefore, it is important to adjust the diet reasonably, advocating for a "high protein, high vitamin, low fat" diet to maintain a balanced nutrition. Regularly review routine blood tests, and if a decrease in white blood cells is found, there is a risk of infection. In such cases, it may be necessary to temporarily pause radiotherapy and follow the doctor's advice for symptomatic supportive treatment.

Supplementary 9 Guidelines for Target Volume Delineation

Clinical practice involves the use of RNI+WBI(BCS)/CWI (complete mastectomy) in patients who are of low-risk clinically but high-risk in terms of RecurIndex LR.

1.1 Whole Breast Clinical Target Volume (CTV_2)

1. Purpose: Applicable for patients who undergo breast conservation surgery and axillary lymph node dissection.
2. Target definition: The entire or majority of the mammary gland on the affected side, encompassing the entire tumor bed target range, entire retro mammary space and pectoralis major fascia, Rotter's lymph nodes in the pectoralis major and minor inter-space, and the mid and lower axilla within the entire breast target range that was not cleared.
3. Target boundaries: • The upper boundary is the palpable/CT-visible upper edge of the gland. • The lower boundary is the palpable/CT-visible lower edge of the gland. • The anterior boundary is 5mm subcutaneously, or 0.3cm subcutaneously or even up to the skin for thin and small breasts. • The posterior boundary is 1-2mm behind the surface of the pectoralis major fascia (adjacent to the retro mammary space), not leaving a fatty gap, excluding ribs/intercostal muscles, but including Rotter's lymph nodes and axillary regions I and II if regional lymph nodes are metastatic. • The medial boundary is next to the sternum, at least to the inner edge of the peristernal vessels. • The lateral boundary is the palpable or CT-visible lateral edge of the gland, anterior to the thoracodorsal artery, anterior edge of the latissimus dorsi muscle.

1.2 Tumor Bed and CTV_1

1. Purpose: Applicable for patients undergoing breast-conserving surgery. Boost to the tumor bed is not mandatory and is determined by the policy of each center. Boost is suggested under the following circumstances: positive margin, close margin (invasive cancer or DCIS with a negative margin ≤2mm), and young age (<50 years).
2. Tumor bed definition: The range of the excised tumor and its surrounding tissue cavity, filled with serous effusion or formed tissue.
3. Tumor bed boundaries: Refer to: (1) The location of titanium clips, with a suggestion of placing clips at the left, right, top, bottom, and rear (2) The range of serous effusion, including that within the gland and sub-scar.
4. Tumor Bed CTV_1: Includes the mammary gland and soft tissues 10-15mm beyond the surgical excision of the tumor bed. It is suggested to reduce this for patients undergoing segmental resection to about 10mm. If there is no gland beyond the tumor bed, consider reducing it; for positive margins or presence of EIC or severe ADH, it is crucial to appropriately increase the range.

1.3 Integrated Target Area of the Whole Breast and Lower and Middle Axillary CTV_2

1. Purpose: Applicable for patients undergoing breast conservation surgery plus sentinel lymph node biopsy.
2. Target definition: Whole breast + axillary lymph nodes in zones I and II + Rotter's lymph nodes between pectoralis major and minor muscles.
3. Target boundaries: Whole breast boundaries refer to 5.2.3.1.1 Whole Breast Target; axillary zone I: Anatomically marked by the pectoralis minor muscle, located laterally to the pectoralis minor; axillary zone II: Follows the course of the axillary vein, located posterior to the pectoralis minor including the intermuscular space of pectoralis major and minor (Rotter's

LN).

Criterion	Axillary Zone I	Axillary Zone II	Rotter's Lymph Nodes
Superior Boundary	Where the axillary vessels cross the lateral edge of the pectoralis minor muscle	Where the axillary vessels cross the medial edge of the pectoralis minor muscle	Including the cephalic side of the axillary artery and 5mm above the axillary vein
Inferior Boundary	Where the pectoralis major muscle inserts into the rib	Where the axillary vessels cross the lateral edge of the pectoralis minor muscle	Inferior boundary of axillary zone II
Anterior Boundary	Anterior to the pectoralis major muscle and latissimus dorsi muscle	Anterior to the pectoralis minor muscle	Posterior to the pectoralis major muscle
Posterior Boundary	Anterior to the subscapularis muscle	Rib and intercostal muscles	Anterior to the pectoralis minor muscle
Medial Boundary	Lateral edge of the pectoralis minor muscle	Medial edge of the pectoralis minor muscle	Medial edge of the pectoralis minor muscle
Lateral Boundary	Medial aspect of the latissimus dorsi muscle	Lateral edge of the pectoralis minor muscle	Lateral edge of the pectoralis minor muscle

1.4 Clinical Target Volume for Chest Wall (CTV_CW)

1. Purpose: Applicable to patients who have undergone total mastectomy ± breast reconstruction surgery.
2. Target Definition: The surgical area that may cause intraoperative spread and carry the risk of recurrence after total mastectomy.
3. Target Boundaries • Upper boundary is a clinical marker/0.5-1cm below the clavicle head; • Lower boundary is a clinical marker/below the contralateral breast fold; • Anterior boundary is the skin, excluding lead wire; • Posterior boundary is the intercostal muscle of the rib; • Medial boundary is a clinical marker/at the junction of the sternum and ribs; • Lateral boundary is a clinical marker/ at the anterior edge of the thoracic and dorsal vessels and the latissimus dorsi.
    - Note: ① Includes all scars, with the target area 2cm above and below the scar not reduced; ② Includes postoperative changes observed on CT scan (granuloma and fibrotic changes, barb-like muscle irritation signs).

1.5 Clinical Target Volume for Supraclavicular and Infraclavicular Lymph Nodes (CTV_LN)

1. Purpose: Suitable for group A patients with low clinical risk and high RecurIndex LR risk requiring regional lymph node radiation.
2. Target Definition: Includes supraclavicular area, neck IV region, and infraclavicular lymph nodes (unsurgically treated Level III axillary lymph nodes).
3. Target Boundaries • Upper boundary is below the cricoid cartilage; • Lower boundary is 0.5-1cm below the clavicle head, where the subclavian vein disappears, and connects with the whole breast/chest wall target area; • Anterior boundary is behind the sternocleidomastoid muscle above and behind the pectoralis major muscle below; • Posterior boundary is the posterior edge of the anterior scalene muscle above and the anterior edge of the rib and intercostal muscle below; • Medial boundary is the internal jugular vein above, including the entire scalene muscle gap to the level of the transverse cervical artery and vein, and the junction of the subclavian vein and internal jugular vein below; • Lateral boundary is the

lateral edge of the sternocleidomastoid muscle above and the lateral edge of the pectoralis minor muscle below.

- Note: ① Avoid the operated axillary area (Level I and part of Level II); ② Includes the unsurgically treated axillary Level II area.

1.6 Internal Mammary Lymph Node Area (CTV_IMN)

1. Purpose: Applicable to Group A patients with low clinical risk and high RecurIndex LR, requiring regional lymph node irradiation. Irradiation of the internal mammary lymph node region is not obligatory and is determined by the radiation oncologist.
2. Target area definition: Those at risk of internal mammary lymph node metastasis may benefit from internal mammary lymph node irradiation. It includes the 1st-3rd intercostal spaces, 5mm in all directions from the intrathoracic vessels, the upper boundary infusing into the supraclavicular area, and the lower boundary at the upper edge of the 4th rib.
3. Target area boundaries: • Superior border: Infusion into the supraclavicular area; • Inferior border: Upper edge of the fourth costal cartilage; • Anterior border: Back of the pectoralis major muscle, back of the sternum; • Posterior border: Pleura or 5mm behind the internal mammary vessels; • Medial border: 5mm inside the internal mammary vessels, including the whole space between the sternum and vessels; • Lateral border: 5mm outside the internal mammary vessels, outer edge of the brachiocephalic vessels.
    - Note: ① For high-risk patients, the upper boundary extends to the junction of the internal jugular vein, subclavian vein, brachiocephalic vein, and internal mammary vein; ② It is recommended to expand at least 5mm inside and outside the direction of the internal mammary vessels.

1.7 Intraclavicular Lymph Nodes (CTV_intraclavicular-LN)

1. Purpose: Applicable to Group A patients with low clinical risk and high RecurIndex LR, requiring regional lymph node irradiation. Irradiation of the intraclavicular lymph node area is not obligatory and is determined by the radiation oncologist.
2. Target area definition and boundaries: Those at risk of intraclavicular lymph node metastasis may benefit from intraclavicular lymph node irradiation. The upper boundary is the horizontal level of the transverse cervical artery, the lower boundary is the upper edge of the brachiocephalic trunk, the medial boundary is the midline of the body, and the lateral boundary is the inner edge of the supraclavicular area.

- Note: ① When irradiating the internal mammary lymph nodes, it should be routinely outlined; ② When there is metastasis of supraclavicular lymph nodes, it should be routinely outlined; ③ When there is extracapsular invasion of axillary Level II/III lymph nodes, it should be routinely outlined; ④ In patients with primary tumors invading the deep fascia, or the primary lesion located in the medial and upper part of the breast, consider outlining.

In patients who are clinically low-risk but exhibit a high-risk RecurIndex LR, regional nodal irradiation (RNI) is omitted, with whole breast irradiation (WBI) after breast-conserving surgery (BCS) only and no chest wall irradiation (CWI) after total mastectomy.

2.1 Entire Breast Target Volume (CTV_2):

1. Purpose: Suitable for patients who have undergone breast conservation surgery with axillary lymph node dissection.
2. Target Definition: This includes all or most of the glandular tissue of the affected breast, encompassing the entire tumor bed, the entire space posterior to the breast and the pectoral

fascia, the interpectoral space (Rotter's lymph nodes), and any unswept low-mid axilla within the entire breast target volume.

3. Target Borders: • Superior border is the palpable/visible edge of the glandular tissue on CT. • Inferior border is the palpable/visible lower edge of the glandular tissue on CT. • Anterior border is 5 mm below the skin, but for small or thin breasts, it should be adjusted to 0.3 cm below the skin or even closer. • Posterior border is 1-2 mm behind the pectoral fascia (adjacent to the space below the breast), with no fat gap left and no inclusion of ribs/intercostal muscles. If regional lymph nodes have metastasized, include interpectoral lymph nodes and unswept axillary regions I and II. • Medial border is next to the sternum, extending at least to the inner edge of the peristernal vessels. • Lateral border is the palpable or CT-visible outer edge of the glandular tissue, anterior to the dorsal thoracic artery, anterior edge of the latissimus dorsi muscle.

2.2 Tumor Bed and CTV1:

1. Purpose: Suitable for patients who have undergone breast-conserving surgery. Boosting of the tumor bed is not mandatory and depends on the policy of each center. Boosting of the tumor bed is recommended under the following conditions: positive surgical margins, close margins (invasive cancer or DCIS with negative margins ≤2 mm), young age (<50 years).
2. Tumor Bed Definition: The area where the tumor and surrounding tissue have been excised, filled with serum exudate or plastic tissue.
3. Tumor Bed Borders: • Based on the location of the titanium clips. It is suggested that titanium clips be placed in five directions: left, right, up, down, and behind. • The extent of serum exudate, taking into account any within the glandular tissue and beneath the scar.
4. Tumor Bed CTV_1: • Includes the resected tumor bed extended by 10-15 mm into the glandular breast tissue and soft tissue. For patients undergoing segmental resection, this may be appropriately reduced, with a suggested 10 mm. If there's no glandular tissue outside the tumor bed, consider reducing it. In case of positive margins or presence of extensive intraductal component (EIC), severe atypical ductal hyperplasia (ADH), the range must be appropriately expanded.

2.3 Integrated Target Volume of the Entire Breast and Low-to-Mid Axillary Region (CTV_2):

1. Purpose: Suitable for patients who have undergone breast conservation surgery with sentinel lymph node biopsy.
2. Target Definition: The entire breast + lymph nodes in axillary regions I and II + interpectoral lymph nodes (Rotter's lymph nodes).
3. Target Borders: • For the entire breast, refer to Section1.1 Entire Breast Target Volume; Axillary Region I: anatomically marked by the small pectoral muscle, located on the lateral side of the muscle; Axillary Region II: follows the axillary vein, located posterior to the small pectoral muscle, and also includes the interpectoral space (Rotter's LN).

Boundary	Axillary Region I	Axillary Region II	Rotter's Lymph Nodes
Superior Border	Where axillary vessels cross the lateral edge of the small pectoral muscle	Where axillary vessels cross the medial edge of the small pectoral muscle	Including the cranial side of the axillary artery and 5 mm above the axillary vein
Inferior Border	Where the large pectoral muscle inserts into the rib	Where axillary vessels cross the lateral edge of the small pectoral muscle	Inferior border of axillary region II

Anterior Border	In front of the large pectoral and latissimus dorsi muscles	In front of the small pectoral muscle	Behind the large pectoral muscle
Posterior Border	In front of the subscapularis muscle	Ribs and intercostal muscles	In front of the small pectoral muscle
Medial Border	Outer edge of the small pectoral muscle	Inner edge of the small pectoral muscle	Inner edge of the small pectoral muscle
Lateral Border	Inner surface of the latissimus dorsi muscle	Outer edge of the small pectoral muscle	Outer edge of the small pectoral muscle

Supplementary **10** Dose distribution and organ endangerment limits

Target Volume	Dmax	Dmin
Whole Breast PTV_2	≤107%	≥90%
Tumor Bed PTV_1	≤107%	≥90%
Whole Breast and Low-to-Mid Axilla Integrated Target Volume PTV_2	≤107%	≥90%
Chest Wall PTV_CW	≤110%	≥90%
Supraclavicular (±intranodal clavicular) PTV_LN	≤110%	≥90%
Internal Mammary PTV_IMN	≤110%	≥80%

Supplementary 11. Organ dose/volume/impact data for routine split exposures (except where noted): QUANTEC

Organs	Volume	Type of irradiation (partial organ or specially indicated)	Observation index	Dose (Gy) or dose volume parameter	Incidence (%)	Dose volume parameter description
Spinal Cord	Partial spinal cord Thoracic medulla	3DCRT	Spinal cord lesions	Dmax=50	0.02	Includes all spinal cord cross-sections
Pharynx	pharyngeal constrictor muscle	3DCRT	Dysphagia and shortness of breath	Dmean＜50	＜20	
larynx	Total larynx	3DCRT	Edema	Dmean＜44	＜20	No chemotherapy, based on a single study of patients without laryngeal cancer
	Total larynx	3DCRT	Edema	V50＜27%	＜20	
lung	whole lung	3DCRT	Pneumonia	V20≤30%	＜20	Double lung. Slow dose response Without whole lung treatment irradiation
	whole lung	3DCRT	Pneumonia	Dmean=7	5	
	whole lung	3DCRT	Pneumonia	Dmean=13	10	
	whole lung	3DCRT	Pneumonia	Dmean=20	20	
	whole lung	3DCRT	Pneumonia	Dmean=24	30	
	whole lung	3DCRT	Pneumonia	Dmean=27	40	
Esophagus	Whole Esophagus	3DCRT	≥3 Grade acute esophagitis	Dmean＜34	5-20	Contains various dose limiting factors. Seems to be related to dose volume
	Whole Esophagus	3DCRT	≥ grade 2 acute esophagitis	V35＜50%	＜30	
	Whole Esophagus	3DCRT	≥ grade 2 acute esophagitis	V50＜40%	＜30	
heart	Pericardium	3DCRT	pericarditis	Dmean＜26	＜15	Based on individual studies
	Pericardium	3DCRT	pericarditis	V30＜46%	＜15	
	Whole heart	3DCRT	distant cardiac	V25＜46%	＜1	High standards for

			death			assessing security based on predictive models
Liver	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 30-32	＜5	Exclude patients with existing liver disease or liver cancer
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 42	＜50	Patients with liver disease or hepatocellular carcinoma with a Child-Pugh rating of A, but not active hepatitis B, were included as observation indicators
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 28	＜5	
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 36	＜50	
	Whole stomach	3DCRT	Ulcer	D100＜45	＜7	

QUANTEC: Quantitative Analysis of Illumination Response in Clinically Normal Tissues; 3DCRT: Three-Dimensional Conformal Radiotherapy; GTV: Gross Tumor Volume; RILD: Radioactive Liver Injury; RTOG: Radiation Therapy Oncology Group of Amer

Supplementary 12. Research Schedule

Research Phase	Screening	Radiotherapy period				Follow up period										
Visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Time	-3M-0	1W	2W	3W	4-7W	6M	12M	18M	24M	30M	36M	42M	48M	54M	60M	In the event of a relapse
Informed consent	×															
Inclusion/exclusion criteria	×															
Frozen/paraffin tissue	×															
Pathology	×*															
Tumor markers	×					×	×	×	×	×	×	×	×	×	×	×
Breast + LN ultrasound/MR	×					×	×	×	×	×	×	×	×	×	×	
Mammography	×*						×		×		×		×		×	
Chest X-ray/CT	×					×	×	×	×	×	×	×	×	×	×	×
Abdominal ultrasound/CT	×					×	×	×	×	×	×	×	×	×	×	×
Thyroid + LN ultrasound	×					×	×	×	×	×	×	×	×	×	×	
Bone Scan	×*						×		×		×		×		×	×
Cranial CT/MRI																×***
Demographic features	×															
Medical history	×															
Physical examination	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	

Upper and lower arm circumference measurement◆	×				×		×		×		×		×		×	
Body weight	×				×		×		×		×		×		×	
ECOG score	×				×		×		×		×		×		×	
Blood count	×**	×	×	×	×	×	×	×	×	×	×		×	×	×	×
Liver and kidney function	×**				×	×	×	×	×	×	×		×	×	×	×
Infectious disease	×*															
Serum pregnancy test	×**															
EORTC QLQ-C30	×**				×		×		×		×		×		×	
EORTC QLQ-BR23	×**				×		×		×		×		×		×	
Harris	×**						×		×		×		×		×	
Baker	×**						×		×		×		×		×	
CTCAE V4.03	×**	×	×	×	×		×	×	×		×		×		×	
Acute radiation reaction		×	×	×	×											
Late radiation injury							×	×	×		×		×		×	

◆Upper and lower arm circumference measurements: measured at the bilateral metacarpophalangeal joints (mid-metacarpal), at the wrist, and at the distal 10 cm and proximal 15 cm of the elbow epicondyle.

* Within 12 months prior to randomization

** Within 14 days before randomization

*** When cranial symptoms were present

Supplementary 13. Physical status ECOG scoring criteria

ECOG scoring criteria	Scoring
Mobility is completely normal and does not differ in any way from that before the onset of the disease	0
Can walk freely and perform light physical activities, including general housework or office work, but cannot perform heavier physical activities	1
Able to walk freely and take care of themselves, but have lost the ability to work, and can get up and move around at least half of the time during the day	2
Only partially able to take care of themselves, bedridden or wheelchair bound for more than half of the day	3
Bedridden and unable to care for themselves	4
Death	5

Supplementary 14. Trial registration data

Data category	Information
Primary registry and trial identifying number	Line 44 page 4
Date of registration in primary registry	7 July. 2022
Source(s) of monetary or material support	Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
Primary sponsor	Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
Secondary sponsor(s)	the Jiangsu Simcere Pharmaceutical Co., Ltd., Jiangsu Simcere Diagnostics Co., Ltd
Contact for public queries	Xiaobo Huang, MD. [huangxbo@mail.sysu.edu.cn]
Contact for scientific queries	Xiaobo Huang, MD. Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China
Public title	**RIGAIN Study**
Scientific title	Line 3 page 3
Countries of recruitment	Line 29 page 4
Health condition(s) or problem(s) studied	Regional lymph node irradiation
Intervention(s)	Line 26 page 4
Key inclusion and exclusion criteria	*Table 1* Line 3 page 18
Study type	Line 27 page 4
Date of first enrolment	Line 38 page 4
Target sample size	Line 33 page 4
Recruitment status	Recruiting
Primary outcome(s)	Line 19 page 8
Key secondary outcomes	Line 19 page 8

Supplementary 15. Statistical Analysis

1. Population Analysis

Full Analysis Set (FAS): Efficacy analysis will be conducted for all cases randomized according to the intention-to-treat (ITT) principle, and analyzed based on their randomized groups, regardless of the actual radiation therapy group received.

Per-Protocol Set (PPS): Subjects from the FAS set will be excluded if they have major protocol violations that could potentially affect the primary efficacy endpoint IDFS analysis. Efficacy evaluation for this study will be conducted for both FAS and PPS sets, with FAS serving as the primary analysis set.

Safety Set (SS): All subjects randomized and receiving at least one session of radiation therapy belong to the safety analysis set. This dataset utilizes actual radiation therapy groups and is used for safety analysis.

2. Demographic and Baseline Characteristics

Descriptive statistical analysis will be conducted for demographic characteristics such as age, gender, height, weight, as well as other baseline features including medical history.

3. Participant Distribution

Descriptive statistical analysis will be performed on participant enrollment status, study completion status, premature study withdrawal, etc. A tabular summary will outline the distribution of participants across different analysis populations.

4. Efficacy Analysis

Efficacy analysis will be based on both FAS and PPS, with FAS serving as the primary analysis set.
Primary Endpoint Analysis: Invasive Disease-Free Survival (IDFS) serves as the primary efficacy endpoint of this study, defined as the time from randomization to the earliest occurrence of invasive cancer local recurrence, distant metastasis, or death, whichever comes first. The occurrence of invasive disease recurrence will be determined by the assessment results obtained by an independent review committee using pathological or imaging examinations. Patients who have not experienced invasive cancer local recurrence, distant metastasis, or death will have their last follow-up date considered as the censoring date. Patients who have not undergone imaging follow-up after baseline will have the randomization date considered as the censoring date. Cox proportional-hazards model will be used for the analysis of the primary endpoint to calculate the Hazard Ratio and its 95% confidence interval, adjusting for stratification factors and other covariates. Additionally, a Cox proportional-hazards model without covariates will be used to support the analysis results of the primary endpoint. Furthermore, the median invasive disease-free survival for both groups (Group A vs. Group B) will be calculated using the Kaplan-Meier (KM) method, including the 95% confidence interval. KM plots will be used to illustrate the trend of IDFS over time.
Secondary Endpoint Analysis: The analysis will compute the Annual Failure Rate (AFR) for each group, along with the corresponding 95% Clopper-Pearson confidence interval. Local recurrence-

free survival (LRFS), distant metastasis-free survival (DMFS), relapse-free survival (RFS), overall survival (OS), disease-free survival (DFS), and breast cancer-specific mortality (BCSM) will be analyzed using the KM method to determine the median values (including the 95% confidence interval). Changes in total scores of EORTC QLQ-C30 and EORTC QLQ-BS23 from baseline will also be summarized.

5. Safety Analysis

Safety analysis will be based on the Safety Set (SS).

The safety analysis will include all enrolled patients who have received at least one session of radiation therapy, grouped according to the actual treatment received by the patients.

Safety will be assessed by summarizing adverse events, changes in laboratory test results, vital sign changes, and exposure to study treatment, reported by treatment group.

All adverse events (AEs) will be classified according to the Medical Dictionary for Regulatory Activities (MedDRA) terminology and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC) Acute Radiation Morbidity Scoring Criteria (1995), and RTOG/EORTC Late Radiation Morbidity Scoring Criteria (1995). All adverse events occurring during treatment (Treatment Emergent Adverse Events, TEAEs, defined as events occurring within 30 days after the end of the last radiation therapy session), TEAEs of Grade 3 or higher, serious adverse events (SAEs), radiation-related SAEs, and TEAEs leading to trial discontinuation will be summarized by system organ class, preferred term, and group in terms of number and percentage. Additionally, the severity and relatedness of TEAEs to radiation therapy will also be summarized by system organ class, preferred term, and group. If a patient experiences the same adverse event multiple times, the maximum reported severity will be used for summarization.

6. Exploratory Studies

Exploratory studies will investigate the relationship between peripheral blood T lymphocyte subsets and the immunomodulatory effects of radiotherapy, as well as the relationship between circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and distant tumor eradication effects.

7. Interim Analysis

Interim analysis is not planned for this trial.

8. Final Analysis

The final analysis for this study is planned to be conducted when the last enrolled patient completes radiotherapy and reaches a follow-up of 5 years.

Supplementary 16. **Data Management**

1. Source Data Recording

Monitoring personnel will verify source data to confirm that the data entered by authorized personnel at the trial center into the electronic Case Report Form (eCRF), i.e., the source data, is correct, complete, and indeed originated from the source documents.

Source documents (paper or electronic) refer to patient data recorded at the earliest time and include but are not limited to: hospital records, laboratory records, memos, patient-reported outcomes, assessment checklists, data recorded on automated instruments, microfiche, photographic films, X-rays, etc.

Source documents requiring verification of data integrity and validity must not be altered or destroyed and must be retained in accordance with the applicable regulatory retention policies.

For source data verification, investigators and trial institutions must provide monitoring personnel with direct access to relevant source documents and reports for audit purposes and Institutional Ethics Board/Ethics Committee (IEB/EC) review. Trial centers must also allow regulatory authorities to conduct inspections.

2. Use of Computer Systems

When clinical observation results are entered directly into the computerized medical record system of the study center, electronic records may be considered as source documents if the system has been validated according to regulatory requirements for computerized systems adopted in clinical research. Original data should be saved using appropriate computerized data collection systems. If original data requires modification, the system must retain visual inspection audit trails showing the original data and reasons for modification, along with the names and dates of modification.

3. Case Report Forms

This study utilizes electronic Case Report Forms (e-CRFs) for data collection, completed using the Electronic Data Capture (EDC) system specified by the principal investigator. The designated vendor appointed by the principal investigator will provide training to trial centers and a suitable e-CRF completion manual to the research centers.

All e-CRFs are completed by designated, trained personnel at the trial center, and the investigator or designated personnel must review, electronically sign, and date the e-CRFs.

4. Data Quality Assurance

The clinical trial office where the principal investigator is located is responsible for data management for this study, including monitoring/audit of data quality. Clinical research data will be collected via eCRFs using the EDC system. Data entry into the ECD system will be the responsibility of the research center. In case of discrepancies, the clinical trial office where the principal investigator is located will request explanations from the research center, and this process will be electronically resolved within the EDC system.

The sponsor will develop an EDC study quality standards document outlining methods for quality checks on the data.

Data from center laboratories will be sent directly to the principal investigator, who will process and electronically transfer these data according to the standard operating procedures recognized by the

principal investigator for center laboratories.

e-CRFs and correction documents will be retained in the EDC system during the auditing process. The data retained by the principal investigator will be systematically backed up according to standard operating procedures recognized by the principal investigator for the vendor, and records of research data retention will be kept.

5. Independent Data Safety Committee

A Data Safety Committee will be established, consisting of an odd number of members (typically 5), who are independent of the project team and have signed confidentiality agreements. The committee will primarily conduct a review analysis of positive results (subject relapse and metastasis) and understand the actual study results (without statistical analysis) when half of the subjects are enrolled, voting on whether adjustments to the study protocol are necessary

Supplementary 17. **Quality Management Plan**

1 Quality Control

1) Qualifications of Study Personnel: All personnel involved in this trial, including investigators, nurses, statisticians, clinical trial observers, etc., must undergo clinical trial training and work under the guidance of senior professionals.
2) Investigators and other personnel involved in the study should fulfill their responsibilities and strictly adhere to the clinical trial protocol, employing standard operating procedures to ensure the implementation of quality control and quality assurance systems.
3) All observed results and findings in the clinical trial should be verified, and quality control must be conducted at every stage of data processing to ensure data integrity, accuracy, authenticity, and reliability.
4) Investigators and other personnel involved in the study should have sufficient time and reliable sources of subjects for conducting the study.
5) All projects involving imaging and laboratory testing should be carried out by units that comply with national standards.
6) Specimens requiring collection in this study should be collected by designated individuals, and follow-up data should be collected and stored by designated personnel.
7) Testing procedures should be conducted according to the specified Standard Operating Procedures (SOPs).
8) When modifications to the study protocol are required, the Ethics Committee should be convened according to SOPs, fully utilizing the Ethics Committee's functions to ensure the protection of subjects' interests.
9) Each participating unit should establish a file folder, save all original materials as required in the protocol, and arrange them in chronological order for verification purposes.
10) Contract research organizations must appoint trained monitors for the study, who should have relevant medical and pharmaceutical backgrounds, and conduct inspections of the research projects according to SOPs (including: pre-trial visits, initiation visits, routine monitoring visits, and end-of-study visits; see "Monitoring Plan").
11) Inspectors should systematically examine clinical trial-related activities and documents to assess whether the trial is conducted in accordance with the protocol, SOPs, and relevant regulatory requirements, and whether trial data are recorded in a timely, truthful, accurate, and complete manner. Inspections should be conducted by personnel not directly involved in the clinical trial.
12) The establishment of inspection work is to ensure that clinical trials are conducted in accordance with the requirements of the protocol, SOPs, and relevant regulations. Contents include: a) How is the clinical research operated? b) Is the implementation in line with the requirements of the study protocol? c) Is the principal investigator effectively and appropriately monitoring the progress of the study? d) How is the quality of the study: whether the study personnel, study centers, and data trial centers adhere to the requirements of SOPs? e) Are the data copied onto the Case Report Form (CRF) consistent with the original data? f) Overall trial quality (identifying the root causes of issues). g) Are study documents present? Are they stored systematically? Are they interpretable (can trial data be reconstructed from study documents)? h) Inspection of monitoring reports attempts to identify quality trends and consult on corrective measures for procedural issues that

have arisen.

13) Regular inspections, preparation of inspection reports, and holding meetings with relevant personnel to discuss issues identified during the inspection.

2 Monitoring Plan

The monitor conducts three types of visits: study initiation visits, routine visits, and close-out visits.

2.1 Study Initiation Visits

Meet with the principal investigator, establish a visit plan, and introduce the monitoring objectives and plans to the investigator. Review includes: training manuals, forms, study protocols, qualifications of participating researchers, and compliance with data management SOPs, etc. If necessary, a start-up meeting can be convened to discuss the protocol and work content with all doctors and other staff participating in the study, clarify each person's responsibilities, explain the SOP requirements for data entry standards, and the preservation of original data.

2.2 Routine Visits

① Before each visit, review the progress of the trial and unresolved issues from previous visits, contact the investigator to confirm the visit date, develop a plan and agenda for this visit, prepare the required documents and items for the visit.

② Meet with the investigator to explain the main tasks of this visit, understand the progress of the trial (subject enrollment status, CRF completion status, informed consent signing status, etc.), and the resolution of problems identified during previous visits.

③ Check and update the investigator's management files, verify the original documents and CRF forms (pay attention to compliance, completeness, consistency with the protocol, discovery, and reporting of SAEs), check trial materials (storage conditions, distribution and recovery records, compliance with protocol requirements).

④ Collect CRF forms.

⑤ Record any issues discovered, discuss and resolve the problems identified during this visit with the investigator, exchange progress and experiences with other research units.

⑥ Store items retrieved, signed informed consent forms, CRF forms, etc., as required.

⑦ Complete the visit report, update various records, track and resolve any issues discovered, and schedule follow-up visit plans.

⑧ SAEs that occur during the clinical trial must be reported to the Ethics Committee within 24 hours.

⑨ Any changes to the protocol, CRF forms, etc., during the trial require approval from the Ethics Committee. Documents to be submitted to the Ethics Committee during the trial include: protocol amendments, informed consent form amendments, SAE reports, recruitment advertisements (if used).

2.3 Close-Out Visits

① Review any outstanding issues from routine visits and confirm their resolution.

② Confirm the visit time, develop a plan and agenda for this visit.

③ Confirm the completeness and updating of the investigator's management files.

④ Confirm that all CRF forms have been collected.
⑤ Confirm the reporting and tracking of SAEs.
⑥ Check the records of the transport, distribution, and retrieval of various materials for the study.
⑦ Discuss and summarize, confirm any outstanding issues and follow-up work, explain the requirements for the preservation of trial-related documents.
⑧ Follow-up work: Complete the trial close-out monitoring visit report, notify the Ethics Committee of the trial's conclusion, continue to track and resolve any outstanding issues, and archive all documents. Documents to be submitted to the Ethics Committee after the trial ends include: trial closure letter, SAEs after trial closure.

3 Data Requirements

Protocol: After careful reading and agreement, the principal investigator must sign and strictly adhere to the protocol implementation.

Clinical Trial Data: All various original clinical trial data should be recorded promptly, truthfully, accurately, and completely, and copies of laboratory test reports should be retained. The principal investigator must retain records and documents of the study implementation process, including eCRFs, informed consent forms, laboratory test results, and radiotherapy plans, for 5 years after the completion or termination of the study, or for a longer period as required by regulatory authorities (whichever is longer). After this time period, the documents may be destroyed in accordance with local regulatory requirements.

4 Study Summary

Once the required total number of cases is reached and verified, the data analysis center performs data analysis. Based on the statistical analysis report, the responsible unit of the clinical trial and participating units write a summary of the clinical trial and sub-center summary table according to the principles of Good Clinical Practice (GCP) clinical trial guidance.

5 Research Funding

The RecurIndex-related testing expenses for this trial are provided by the collaborating party, with specific funding arrangements outlined in a signed contract.

6 Financial Transparency

The principal investigator is required to provide complete and accurate financial information in accordance with Chinese regulations, in order to submit comprehensive and accurate financial statements or disclosure statements to relevant health authorities. The principal investigator is responsible for providing financial information from the beginning to the completion of the study period.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (*pg3. line 3*)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (*pg4. line 44*)
	2b	All items from the World Health Organization Trial Registration Data Set (Supplemental 14)
Protocol version	3	Date and version identifier (*pg4. line 42*)
Funding	4	Sources and types of financial, material, and other support (*pg14. line 43*)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (*pg3. line 9*)
	5b	Name and contact information for the trial sponsor (*pg3. line 54*)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities. (*pg14. line 33*)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (*pg4. line 60*)
	6b	Explanation for choice of comparators (*pg5. line 20-43*)
Objectives	7	Specific objectives or hypotheses (*pg8. line 14*)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (*pg4. line 26*)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (*pg6. line 59*)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (*pg7. line 7*)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (*pg7. line 17 and pg9. line 52*)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) (*pg10. line 43*)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (*pg7. line 52*)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Not available)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (*pg8. line 19*)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Table 3)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (*pg11. line 41*)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (*pg7. line 52*)
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions (*pg7. line 41*)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned (*pg7. line 45*)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (*pg7. line 52*)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Not applicable)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (Not applicable)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol (*pg11. line 20*)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols (*pg10. line 40*)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol (*pg11. line 22*)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Supplementary 15)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Supplementary 15)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) (Supplementay15)

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed (Supplementary 16)
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial (Supplementary 15)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct (Supplementary 15)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (Supplementary 16)

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (*pg12. line 35*)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) (*pg12. line 45*)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial (*pg12. line 48*)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (*pg14. line 51*)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators (Supplementary 16)
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions (*pg4. line 44*)
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates ("Informed Consent Form for RCT" and "Informed Consent Form for Tumor Tissue Biopsy")
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable (Supplementary 17)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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BMJ Open

RecurIndex-Guided postoperative radiotherapy with or without Avoidance of Irradiation of regional Nodes in 1-3 node-positive breast cancer (RIGAIN): a study protocol for a multicenter, open-label, randomized controlled prospective phase III trial

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RecurIndex-Guided postoperative radiotherapy with or without Avoidance of Irradiation of regional Nodes in 1-3 node-positive breast cancer (RIGAIN): a study protocol for a multicenter, open-label, randomized controlled prospective phase III trial

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ABSTRACT

Introduction

Postoperative radiotherapy in breast cancer patients with 1-3 lymph node metastases, particularly within the pT1-2N1M0 cohort with a low clinical risk of local-regional recurrence (LRR), has incited a discourse surrounding personalized treatment strategies. Multigene testing for Recurrence Index (RI) model capably differentiates patients based on their level of LRR risk. This research aims to validate whether a more aggressive treatment approach can enhance clinical outcomes in N1 patients who possess a clinically low risk of LRR, yet a high RecurIndex-determined risk of LRR. Specifically, this entails postoperative whole breast irradiation (WBI) combined with regional lymph node irradiation (RNI) following breast-conserving surgery (BCS) or chest wall irradiation (CWI) with RNI after mastectomy.

Methods and Analysis

The RIGAIN study is a multicenter, prospective, randomized, open-label, phase III clinical trial that is being conducted in China. In this study, patients with low clinical LRR risk but high RecurIndex LRR risk are randomly assigned in a 1:1 ratio to the experimental group or the control group. In the experimental group, RNI is performed and the control group omits RNI. Efficacy and safety analyses will be conducted, enrolling a total of 540 patients (270 per group). The primary endpoint is invasive disease-free survival, and secondary endpoints include any first recurrence, local-regional recurrence-free survival, distant metastasis-free survival, recurrence-free survival, overall survival, disease-free survival, breast cancer-specific mortality, and assessment of patient quality of life. The study began in April 2023 and with a follow-up period of 60 months after the last participant completes radiation therapy.

Ethics and dissemination

The study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-097-02, version 3.1). It adheres to the Helsinki Declaration and good clinical practice. Research findings will be submitted for publication in peer-reviewed journals.

Trial registration number NCT04069884

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The trial is designed as a multicenter, open -label, randomized, controlled phase III study.
- Introduced a multigene model to guide precision radiotherapy.
- This research utilizes IDFS as the primary endpoint.
- The trial is conducted only in one country (China).

INTRODUCTION

Patients with 1-3 axillary lymph node metastases constitute approximately 25 to 30% of early operable breast cancer cases. Radiotherapy plays a pivotal role in the comprehensive treatment of

breast cancer[1,2]. However, the benefit of postoperative radiotherapy for N1 breast cancer patients, particularly in terms of survival improvement, remains a topic of substantial debate. Studies conducted in the 1990s such as the Vancouver study, DBCG-82b/82c, and the early meta-analysis by EBCTCG (including the N1 subgroup) consistently demonstrated that postoperative radiotherapy significantly enhances disease-free survival (DFS) and overall survival (OS) for patients[3-7]. Consequently, N1 becomes a relative indication for postoperative radiotherapy. The 2011/2014 EBCTCG meta-analysis further suggested that postoperative radiotherapy could convert a 1.5% reduction in the 10-year any first recurrence rate (AFRR) into a 1% 20-year OS benefit[8,9]. The MA20 and EORTC 22922 studies published in 2015[10,11], which focused on T1-2N1 patients, especially those with high clinical risk of LRR and compared postoperative RNI after BCS or without RNI, found that more aggressive postoperative RNI for T1-2N1 patients could result in better distant metastases-free survival (DMFS), DFS, or breast cancer-specific mortality (BCSM). The Vancouver study's 20-year long-term follow-up results demonstrated the long-term OS benefit of postoperative radiotherapy in the N1 subgroup. These milestone studies further reinforced the value and recommendation of postoperative radiotherapy for N1 breast cancer patients, rendering N1 staging a strong relative indication for postoperative radiotherapy and increasing the number of patients actively accepting postoperative radiotherapy. Nevertheless, not all N1 patients will benefit from postoperative radiotherapy. Some real-world retrospective studies reveal limited LRR and/or survival improvement from postoperative radiotherapy, particularly RNI therapy, among certain N1 patients, especially those with relatively low clinical risk. Consequently, the necessity of radiotherapy for clinically low LRR risk N1 patients remains a topic of significant controversy and uncertainty. Clinical practice often presents varying professional recommendations for postoperative radiotherapy in low-risk N1 patients, resulting in the exclusion of a substantial number of patients solely based on traditional clinical and pathological features. However, this omission of RNI could lead to inadequate treatment, with potential implications for tumor recurrence, metastasis, and patient survival. Conversely, a uniform approach of postoperative radiotherapy for all clinically low LRR risk N1 patients would inevitably result in overtreatment and expose patients to additional risks such as radiation-induced injury and related complications, thereby impacting their quality of life[12-15].

When considering low-risk N1 breast cancer patients, then, the primary objective is to identify the actual high-risk patients concealed within the clinically low-risk population and to strategically administer postoperative radiotherapy. This represents one of the essential development directions of early breast cancer "precision radiotherapy" in the future. Achieving individualized and precise radiotherapy depends on the discovery of molecular genetic prediction models that can accurately predict local-regional recurrence risk in a scientific, reliable, and accessible manner. Currently approved multi-gene detection models abroad include Oncotype DX, MammaPrint, and EndoPredict. Oncotype DX is the most representative and extensively utilized multi-gene prognostic analysis method, primarily employed to guide early luminal low-risk patients to avoid adjuvant chemotherapy. Oncotype DX is currently more frequently used in the radiotherapy field to identify low-risk elderly N0 breast-conserving patients exempt from postoperative radiotherapy. Although the predictive value in the N1 population has initially

demonstrated some clinical significance, contradictions exist between various research findings[16-19]. No prospective high-level evidence for multi-gene models predicting RNI benefits in N1 patients is currently available. The clinical trial Tailor RT (MA 39) conducted by the Canadian Cancer Trials Group primarily investigates whether low-risk recurrence patients can be spared from postoperative radiotherapy or RNI. This is currently the only prospective, randomized, controlled phase III study internationally that utilizes a multi-gene predictive model to guide precise radiotherapy for N1 patients. The future research outcomes will primarily be applied to guide the omission of postoperative radiotherapy in clinically low LRR risk and genetically low-risk N1 patients. However, the significance of postoperative radiotherapy for patients with intersecting risks, especially those with clinically low-risk but genetically high-risk profiles, remains uncertain.

RecurIndex is the only risk prediction model developed based on the Chinese population for early-stage breast cancer. Consisting of 18 core genes and 10 IHC4 reference genes, it is capable of independently predicting the risk of LRR and distant metastasis[20-24]. Internal validation studies in Taiwan and external validation studies conducted in Singapore, Hong Kong, and the Fourth Affiliated Hospital of Hebei Medical University in China have all provided strong evidence of RecurIndex's predictive efficacy and its value in guiding radiotherapy for N1 patients[25,26]. Low-risk and high-risk patients identified by RI-LRR had 5-year LRR rates of 0% and 7%, respectively (P=0.0146). Compared to high-risk RI-LRR patients who did not receive postoperative radiotherapy, those who underwent postoperative radiotherapy demonstrated significantly improved rates of LRR and RFS, with percentages of 88.8% vs 74.1% (P=0.0071) and 79.4% vs 59.5% (P=0.0019), respectively. These results clearly indicate the significant benefits of postoperative radiotherapy in this patient population. To date, RecurIndex has become widely recognized and clinically implemented around the Asia-Pacific region. It has been incorporated into the "Expert Consensus on Multigene Testing for Adjuvant Therapy of Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Early Breast Cancer" in China and recommended in the "Chinese Society of Clinical Oncology (CSCO) Guidelines for the Diagnosis and Treatment of Breast Cancer 2022" for guiding precise postoperative radiotherapy in N1 patients. However, further high-level, randomized, controlled phase III clinical trials are needed to validate its clinical applications and expand its usage in the field. The most promising and crucial area for its application lies in guiding precise radiotherapy for N1 breast cancer patients.

In summary, we have begun conducting a multicenter, prospective, randomized, controlled phase III clinical study of individualized precision radiotherapy for clinically low LRR risk breast cancer patients with N1 guided by RecurIndex. This study aims to evaluate patients' local recurrence and distant metastasis risks, primarily investigating whether active postoperative radiotherapy can further improve clinical efficacy in N1 patients with clinically low risk but high RecurIndex LRR risk. The ultimate goal of this study is to provide high-level clinical evidence and reliable multi-gene recurrence risk prediction models to help achieve individualized precision radiotherapy for N1 breast cancer patients.

MATERIALS AND METHODS

The RIGAIN study is a multicenter, prospective, randomized, open-label, phase III clinical trial that is being conducted in China. The overall research process is illustrated in Figure 1. This study aims to screen postoperative patients with early-stage breast cancer (pT1-2N1M0) who have completed standard systemic therapy and possess eligible pathological specimens for participation. The inclusion and exclusion criteria are listed in Table 1. RecurIndex testing will be performed using postoperative paraffin-embedded tissue sections from the primary lesion. The study is divided into a randomized controlled trial and an observational study based on clinical risk and RecurIndex LRR risk. Patients with low clinical risk but high RecurIndex LRR risk will be randomly assigned in a 1:1 ratio to either the experimental group (RNI) or the control group (No RNI), while patients with low clinical risk and low RecurIndex LRR risk will be included in the observational study. This article primarily focuses on the randomized controlled trial. The study participants will receive the following treatments. Experimental group: For patients who underwent BCS, RNI will be performed in combination with whole breast irradiation (WBI) + tumor bed boost irradiation. For patients who underwent mastectomy, chest wall irradiation (CWI) will be administered in combination with RNI. Control group: RNI will be omitted. For patients who underwent BCS, only WBI + tumor bed boost irradiation will be administered. For patients who underwent mastectomy, both RNI and CWI will be omitted. A comparative effectiveness analysis will be conducted. The study commenced in April 2023.

Randomization Method

Stratified randomization will be used for the randomized study. For the active postoperative radiotherapy trial involving the clinically low LRR risk but high RecurIndex LRR risk population, participants will be stratified by N1 status, surgical method, and enrolling hospital, and then randomized in a 1:1 ratio into the experimental and control groups.

Randomization stratification factors are as follows:

a) N1 status: N1mic or 1-2 LN macrometastasis (including N1sln), or 3 LN macrometastasis;

b) Surgical method: breast-conserving surgery or mastectomy; and

c) Multicenter enrolling hospital.

A central randomization system was developed by TaiMei Medical Technology Company to facilitate the randomization process. Statistical experts responsible for randomization designed the randomization parameters in advance, allowing the system to generate a random allocation table. The main clinical trial centers conduct eligibility screening for potential participants. Once deemed eligible, the researchers at each sub-center access the server via the internet and enter the information of the enrolled patients. The system then assigns a corresponding randomization number based on the random allocation table, determining the patient's placement in the respective study group.

Participants and recruitment

Patients will be recruited by radiation oncologists from each participating research center. For each interested patient, the clinician or clinical coordinator will provide a complete and comprehensive introduction to them or their designated representative, informing the patient about their rights, the risks involved, and the potential benefits they may receive to enhance their

compliance with the protocol. Prior to enrollment, patients are required to sign an informed consent form, which will be kept in the Case Report Form (CRF). Patient registration is scheduled to begin on April 1, 2023 (See supplementary 1 for details), and is expected to continue for 5 years (tentatively until January 2028). The final collection of data for the primary outcome measures is anticipated to be completed by December 2032.

Patient and public involvement

Neither patients nor the public were involved in the design, conduct, reporting, or dissemination plans of this study.

Objectives and endpoints

This study aims to evaluate whether adjuvant radiotherapy to the regional lymph nodes after breast-conserving surgery or chest wall plus regional lymph node radiotherapy after total mastectomy can further improve clinical outcomes in N1 patients with low clinical risk but high RecurIndex LRR risk.

The primary endpoint is invasive disease-free survival (IDFS). Secondary endpoints are any first recurrence (AFR), local-regional recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), recurrence-free survival (RFS), overall Survival (OS), disease-free survival (DFS), breast cancer-specific mortality (BCSM) and patient quality of life assessment. The specific definitions can be found in Table 2.

During the screening period and 3 months after the end of treatment, patients in each group fill out the quality of life questionnaire (EORTC QLQ-C30), as well as the breast cancer survival quality scale (EORTC QLQ-BS23) annually during the follow-up phase. (Supplementary 2 and 3).

RecurIndex Test

RecurIndex testing was performed using postoperative paraffin-embedded tissue sections from the primary lesion of the subjects. All sections were uniformly sent to the Jiangsu Simcere Pharmaceutical Co., Ltd., Jiangsu Simcere Diagnostics Co. for free testing, Ltd. Formalin-fixed, paraffin-embedded tissue blocks should be selected that cover the largest amount of tumor cells and meet the diagnostic criteria in appearance. Tissue sections with an excess amount of normal tissue, necrotic tissue, adipose tissue, or hemorrhagic tissue should not be sent for examination. The tumor cell content in the identified sections should be >50% for the test to be performed. A total of 10 consecutive sections are needed, each with a thickness of 5 microns. The sections can remain unstained and without coverslips. There is no need to oven-dry the sections; they can be air-dried naturally.

Safety Assessment Indicators

All patients participating in the RIGAIN study are required to undergo safety assessments, including acute radiation reactions and late radiation injuries for radiotherapy patients. The evaluation criteria and handling of injuries are detailed in Supplementary 4-7. Their treatment is shown in Supplementary 8. Including acute skin reactions to radiotherapy, symptomatic radiation pneumonitis, long-term cosmetic outcomes (BCS/reconstruction patients), skin fibrosis (total mastectomy patients), ischemic heart disease, upper limb edema[27,28], brachial plexus injury and

second primary tumor[29].

Radiotherapy

General consideration

The overall treatment plan for each participant is determined by the researchers at the corresponding sub-center based on the participant's condition. Depending on their assigned group, patients will either undergo RNI or be exempted from it. Breast-conserving patients will all receive WBI. Patients should start radiotherapy within 8 weeks after completion of adjuvant chemotherapy. The regional lymph nodes include the supraclavicular lymph nodes and infraclavicular lymph nodes (unresected levels II/III axillary lymph nodes), with or without internal mammary lymph nodes (at least from the 1st to the 3rd intercostal space). For patients with minimally positive SLN and without ALND, the inclusion criteria encompass the low/intermediate axillary lymph nodes. The planned endocrine therapy and anti-HER2 treatment can be continued during the RT process.

Patient positioning and immobilization

The patient lies on a fixed device such as a breast support, vacuum bag, or foam pad. A CT scan is performed with a thickness of 3-5 mm, from the second cervical vertebra to the second lumbar vertebra. CT positioning includes surface marking, where lead wires are placed on the surgical scar of the primary lesion in breast-conserving patients or the chest wall scar in total mastectomy patients, as well as on the scar of the axillary sentinel/clearance lymph node incision. If there is a drainage site, it should also be separately marked with a lead wire or lead point.

Volumes of interest

The clinical target volume (CTV) and organs at risk (OAR) must be delineated on all CT slices, following the contouring guidelines of the Radiation Therapy Oncology Group (RTOG) and considering the actual situation at each CT slice. Detailed descriptions of CTV and OARs can be found in Supplementary 9. The margin between the planning target volume (PTV) and CTV depends on the institutional standards of each participating center, with a recommended minimum of 5 mm. Contours should be drawn according to the RTOG guidelines, including the ipsilateral and contralateral lungs, heart, humeral heads, and spinal cord.

External beam equipment and techniques

Radiation therapy techniques that can be employed include three-dimensional conformal radiotherapy (3DCRT), forward intensity-modulated radiotherapy (F-IMRT), inward intensity-modulated radiotherapy (I-IMRT), volumetric modulated arc therapy (VMAT), and helical tomotherapy (HT). Conventional radiotherapy (using a simulator for positioning and a 2D planning system to design treatment plans with external and tangential fields) and proton therapy techniques are not allowed. Some variations in treatment planning and implementation are permitted to accommodate the participating centers in adapting to the research protocol. However, it is strongly recommended that the treatment plans for enrolled patients at each center remain consistent to avoid confusion.

Dose prescription, fractionation

The whole breast target volume, or the integrated target volume of the whole breast and low to moderate axillary region, or the chest wall target volume, and the regional lymph node target volume receive a radiation dose of 5000 cGy in 25 fractions, delivered at a rate of 200 cGy per day, five days per week. Alternatively, a hypofractionated radiotherapy scheme can be chosen, with a radiation dose of 4000-4256 cGy in 15 to 16 fractions. For breast-conserving patients, a sequential tumor bed boost is performed after completion of whole breast irradiation, as determined by individual center investigators. It can be delivered using conventional fractionation, with a dose of 1000 cGy in 5 fractions at a rate of 200 cGy per day, or by using hypofractionation, with a dose of 798 cGy-1064 cGy in 3 to 4 fractions at a rate of 266 cGy per day. If there are high-risk factors for local recurrence, such as positive surgical margins, close margins, or young age, the radiation dose for the tumor bed boost may be increased to 1400-1600 cGy in 7 to 8 fractions at a rate of 200 cGy per day (See supplementary 10 for details).

DVH constraints

It is required that at least 95% of the prescribed dose to the PTV covers 95% of the PTV. The specific dose distribution is determined by each center's policy, with a recommended level as shown in Supplementary 11. For breast-conserving patients, it is recommended to achieve a central axis dose uniformity of ≤±7% for PTV_2 (whole breast or integrated target volume of whole breast and low to moderate axillary region) and PTV_1 (tumor bed), and to minimize the volume receiving ≥105% of the prescribed dose. The constraints for organs at risk should follow the Quantitative Analysis of Normal Tissue Effects in Clinical (QUANTEC) guidelines (see Supplementary 12).

Withdrawal from research and study termination

Termination of treatment

Research treatment will be terminated if any of the following conditions occur in the patient. The following are the criteria for the withdrawal or dropout of subjects:

1. The subject withdraws informed consent;
2. Any AE causes the subject to be unable to continue participating in the study;
3. The subject is lost to follow-up;
4. The subject does not comply with the study requirements and/or the investigator's instructions;
5. The subject has a concomitant illness or change in the subject's condition, and the investigator believes the subject is no longer suitable for the study treatment; or
6. For any other reason the investigator believes the subject is not suitable for continuing in the study.
7. If a subject drops out or withdraws, relevant safety and efficacy evaluations should be completed as soon as possible.

Study Termination

The trial will be terminated if any of the following situations occur during the trial:

1. Serious safety issues arise during the trial;
2. There is a major error in the study protocol;
3. The principal investigator voluntarily stops the trial; or
4. The administrative authority revokes the trial.

5. The termination of the trial may be temporary or permanent.

If the trial is terminated, all trial records should be retained for review.

Follow-up evaluation and toxicity assessment

The registration timeline, intervention measures, and assessments are presented in Supplementary 13. In the follow-up phase after radiotherapy, check-ups and assessments will be performed every six months until the occurrence of an endpoint event or the end of the study. For patients without postoperative radiotherapy, check-ups and assessments will be conducted every six months after the completion of adjuvant chemotherapy until the occurrence of an endpoint event or the end of the study. Effectiveness evaluations include tumor imaging examinations and assessments, brain MRI or CT, bone scans, quality of life questionnaires (EORTC QLQ-C30), and breast cancer-specific quality of life scales (EORTC QLQ-BS23). Safety evaluations include but are not limited to physical examinations, ECOG PS scores, pregnancy test checks, blood routine tests, blood biochemistry tests, adverse events, and serious adverse events. At the end of the study, participants will undergo physical examinations, performance status assessments (ECOG PS), blood routine tests, blood biochemistry tests, tumor markers, breast ultrasound/MRI, mammography, chest X-ray/CT, abdominal ultrasound/CT, and EORTC QLQ-C30 and EORTC QLQ-BS23 scoring. Evaluations of concomitant medications and adverse events are also required. The end-of-study visit window is 60 months after the last participant completes radiotherapy. For participants who are withdrawn or drop out before the end of the study, safety and effectiveness assessments will be conducted according to the requirements of end-of-study safety and effectiveness visits.

Data Management and Quality Assurance

In this study, electronic case report forms (eCRF) are used to collect data, and the EDC system designated by the principal investigator is used to complete the eCRF. Monitors verify the original data to ensure that the data entered into the eCRF by authorized trial center personnel (i.e., original data) is accurate, complete, and derived from original documents. Researchers and trial institutions must provide monitors with direct access to applicable source documents and reports for inspection and IEB/EC review (See supplementary 14 for details). A Data Safety Committee has also been established, consisting of 5 members who are independent of the project team and have signed a research confidentiality agreement. The main tasks of the committee are to review and analyze positive results (recurrence and metastasis of subjects) and to understand the actual research results (without statistical analysis) when half of the subjects are enrolled. The committee will vote on whether it is necessary to adjust the research plan. This protocol does not include investigational drugs, and any toxicities that occur during treatment should be reported to the principal investigator and their ethics committee. In addition, the sub-centers should also report to the ethics committee of their institution. All serious adverse events and other adverse events must be recorded in the case report form. Furthermore, we have established a comprehensive Quality Control Standard Operating Procedure (SOP), as detailed in Supplementary 15.

Sample Size Estimation

This study is designed for superiority, referring to authoritative postoperative radiotherapy studies MA20[10] and EROTC22922[11] for N1 patients, in which the 5-year IDFS in the

radiotherapy group and the control group were 90.7% vs 81.9% and 87.7% vs 77.1%, respectively. In the domestic RecurIndex external validation retrospective study[21] for N1 breast cancer patients, the 5-year IDFS in the high RI-LRR risk group was 81.1% vs 69.7% in the postoperative radiotherapy group and the control group, respectively. It is expected that the 5-year IDFS for the clinically low LRR risk and high RecurIndex LRR risk population in the experimental group and control group in this study will be 89% and 82%, respectively. The superiority margin is set to improve the primary endpoint IDFS by ≥7% (HR=0.587) in the postoperative radiotherapy research group compared to the control group. With a one-sided significance level (α) of 0.025 and a power (1-β) of 0.8, assuming the experimental group performs better than the control group, the required sample size for each group was calculated as 216 cases per group using PASS15.0 software. The allocation ratio between the experimental and control groups was set at 1:1. Considering a 5-year enrollment period, 5-year follow-up period, and potential 20% dropout rate (mainly considering the need for further 10-year and 15-year long-term efficacy follow-up after reaching the 5-year endpoint), each group will need 270 cases, totaling 540 cases.

Statistical analysis

Descriptive statistical analyses will be conducted based on demographic characteristics such as age, gender, height, weight, and other baseline characteristics such as medical history. The Cox proportional-hazards model will be used for analysis of the primary endpoint. The Hazards Ratio and its 95% confidence interval will be calculated, including stratification factors and other covariates. Additionally, the Cox proportional-hazards model without covariates will be used to support the analysis results of the primary endpoint. Moreover, the Kaplan-Meier (KM) method will be used to calculate the median invasive disease-free survival for the two groups (experimental group vs control group), including 95% confidence intervals. KM plots will be used to illustrate the time trends of IDFS. For the secondary endpoints, the AFR of each group will be calculated, as well as the corresponding 95% Clopper-Pearson confidence intervals. LRFS, DMFS, RFS, OS, DFS, and BCSM will be analyzed for median values using the KM method (including 95% confidence intervals). The overall changes in EORTC QLQ-C30 and EORTC QLQ-BS23 scores from baseline will be summarized. Safety analysis will be conducted by summarizing adverse events, changes in laboratory test results, changes in vital signs, and study treatment exposure. The results will be reported by treatment group. All adverse events during treatment, grade 3 or higher TEAEs, serious adverse events (SAEs), radiotherapy-related SAEs, and TEAEs leading to study termination will be summarized by organ system, preferred term, and group in terms of numbers and percentages (See supplementary 16 for details). A p value ≤0.05 in a two-tailed test will be considered statistically significant. Statistical analyses will be performed using SPSS V.25.0 (Statistical Package for Social Sciences) and STATA V.14.

Ethics and dissemination

This study has obtained approval from the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-097-02, version 3.1), as well as approval from the respective participating centers' ethics committees. The study is being conducted in accordance with the Helsinki Declaration and good clinical practice. Approval from the Chinese Human

Genetic Resources Office was obtained on January 6, 2021, with the reference number 2020SQCJ2358. The study was registered on ClinicalTrials.gov on July 7, 2022, with the registration number NCT04069884. The research findings will be published in peer-reviewed journals. The authors will be individuals who have made significant contributions to the study, design, and implementation. Any modifications to the study protocol and informed consent documents must be reviewed and approved by the Ethics Committee before implementation.

Confidentiality and protection of participants' rights and interests

Researchers are required to explain to participants that participation in the clinical trial is voluntary, and that they have the right to withdraw from the study at any stage without affecting their medical treatment and rights. Personal information of participants will be kept confidential. Participants should be informed about the nature, purpose, potential benefits, and possible risks of the clinical trial, as well as alternative treatment options. Researchers should ensure that the rights and obligations of participants, as stipulated in the declaration, are protected. Participants should be given adequate time to consider whether to participate and to sign the informed consent form.

Discussion

The RIGAIN study is a multicenter, open-label, randomized controlled phase III clinical trial. Our objective is to precisely assess patients with clinically low LRR risk N1 breast cancer who, if identified as high-risk for LRR by the RecurIndex test, may receive enhanced clinical efficacy from active RNI after BCS or CWI with RNI after mastectomy. The aim is to accurately identify patients who would benefit from intensified radiotherapy. In addition, we have established an observational study to investigate the potential to exclude RNI for patients who are clinically low LRR risk and are identified as low risk for LRR by the RecurIndex. The primary endpoint of the study is LRR, with the aim of identifying truly low-risk patients for whom radiotherapy can be safely omitted from planned treatment regimens. Similarly, the MA39 study defines a clinically and genetically low-risk LRR N1 population (age ≥40 years, luminal A type, Oncotype DX score <18) based on comprehensive clinical pathology, molecular subtype, and a multigene model. The anticipated results from the MA39 study could potentially guide personalized RNI decisions for patients who are found to be both clinically and genetically low risk. However, this study also has limitations. Firstly, the multi-gene models used in this study were developed to predict the risk of distant metastasis, and there may be inconsistencies between the occurrence of LRR and the risk of distant metastasis in clinical patients. Secondly, future research results are primarily intended to guide the omission of postoperative radiotherapy in clinically low-risk and genomically low-risk N1 patients. However, the significance of postoperative radiotherapy in patients with intersecting risks, particularly those who are clinically low-risk but genomically high-risk, remains unclear. Lastly, this study does not provide direct evidence for the application of Oncotype DX in guiding treatment decisions for Asian patients.

Traditionally, postoperative radiotherapy has been lauded for decreasing LRR and for helping to diminish the risk of distant metastases[10,11]. This has lead to long-term improvements in DFS and BCSS, providing the ultimate benefit to patients. Notably, this is partly attributed to the prevention of reseeding from recurrences. Another part is attributed to the radiation-induced

abscopal killing effect (RIAKE), which refers to a series of immunological responses induced by local high-dose radiotherapy that culminate in the elimination of tumors distant from the irradiation site[27]. In the context of postoperative radiotherapy for patients with regionally lymph node-positive breast cancer, the abscopal effect is most pronounced in patients classified as pN1, where the survival benefit is most conspicuous[9]. Compared to mastectomy, BCS better preserves the immune microenvironment, thereby enhancing the transformation and activation of the immune response following postoperative radiotherapy. This is the primary reason for our selection of IDFS as the main endpoint in this study.

Oncotype DX and MammaPrint assays primarily assess the overall recurrence risk and mainly guide chemotherapy and endocrine treatment[16,18,30]. Previous studies have indicated a high concordance in predicting the risk of distant metastases between the RecurIndex and the MammaPrint and Oncotype DX assays. However, some discrepancies exist in assessing the risk of LRR. The TAILORx study indicated that the RecurIndex predictive model may identify patients at risk of locoregional recurrence more accurately than the Oncotype DX[16,31,32].

The RecurIndex predictive model stands out amongst various multigene prediction models in early-stage breast cancer with the following unique characteristics and advantages: 1. Unlike other models that only assess overall recurrence risk and are more biased towards the risk of distant metastases, RecurIndex can independently assess both the risk of locoregional recurrence and distant metastases, making it more suitable to guide precision radiotherapy; 2. RecurIndex demonstrates predictive efficacy in populations with HER2 overexpression and triple-negative breast cancer, potentially serving as a precise predictor of locoregional recurrence risk in patients with these two types of N1mic tumors, which could help guide individualized radiotherapy decisions.

The study focuses on the RecurIndex risk prediction model with the aim of guiding postoperative individualized radiotherapy for pT1-2N1M0 breast cancer patients. Particular attention is paid to the "clinically low LRR risk" but "genetically high-risk" population to explore and validate the clinical benefits of postoperative radiotherapy. The study design stands out for its clinical applicability and innovation as well as strict adherence to ethical and clinical practice standards. It effectively addresses the research gap in precise radiotherapy for N1 patients with overlapping risk profiles, both domestically and internationally. The study could potentially revolutionize the practice of postoperative radiotherapy by transitioning from a discretionary approach solely based on clinical and pathological information to an individualized optimization guided by clinical-genetic risk.

We anticipate that the RIGAIN study will generate high-quality evidence, establishing a precise risk assessment framework to guide optimized radiotherapy decisions for N1 breast cancer patients.

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Contributors

XH, YT and ZB designed the original protocol for the study. JC contributed to study management. JL, XH, YT and ZB drafted the manuscript. JL submitted the study. YT and ZB performed the sample size calculation and data analysis. RD and FW offer genetic testing. XH, JL, YT, JC, SH, AZ, LZ, YW, ZL, HY, XX, JC, XW-L, XL, XZ, WZ and XY participated in enrollment, treatment and follow-up of patients.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

Provenance and peer review Not commissioned; externally peer reviewed.

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Table1. Eligibility criteria for the study

Inclusion criteria 1. Age ≥18 years, ≤70 years; 2. ECOG PS ≤2 (Supplementary 17); 3. Postoperative pathology confirms the diagnosis of invasive breast cancer; 4. Meets the clinical definition of low risk: ① Axillary lymph node micrometastasis (N1mic), or ② N1 patients who meet all of the following conditions: a) Age ≥40 years; b) Lymphovascular invasion (LVI) negative or limited to individual or small foci of LVI (excluding extensive or large amounts of LVI); c) Three clinical molecular subtypes (Luminal A type, Luminal B1 type, and Luminal B2 type) are allowed in this study: ER-positive (ER≥1%) and HER2-negative, or ER-positive (ER≥1%) and HER2 overexpressing, respectively. 5. Postoperative pathological diagnosis of axillary lymph node status as any of the following: a. Sentinel lymph node biopsy or axillary lymph node dissection with micrometastasis (N1mic), b. Sentinel lymph node biopsy with 1-2 lymph node macrometastasis (N1sln), c. Sentinel lymph node biopsy + axillary lymph node dissection or simple axillary lymph node dissection with 1-3 lymph node metastasis (N1); 6. The primary tumor and breast underwent breast-conserving surgery or mastectomy ± breast reconstruction (autologous/prosthetic); 7. A thorough systemic examination (e.g., chest X-ray, ultrasound, CT, etc.) within 3 months before randomization for radiotherapy must confirm no distant metastasis; 8. Mammography and/or MRI within 12 months before surgery or randomization for radiotherapy must confirm no contralateral breast cancer; 9. Postoperative completion of at least 4 cycles of adjuvant chemotherapy containing anthracycline or taxane regimens; 10. Radiotherapy must be performed sequentially after the completion of all adjuvant chemotherapy, starting no later than 8 weeks after the end of chemotherapy; 11. Patients must have sufficient postoperative paraffin tissue sections of the primary tumor for RecurIndex testing; 12. No history of other malignant tumors, except for basal cell carcinoma of the skin; and 13. Signed informed consent before the start of the study.
Exclusion criteria 1. Confirmed T3-4, N0, N2-3, M1 stage disease before postoperative radiotherapy enrollment; 2. Received any neoadjuvant treatment before surgery, including chemotherapy, endocrine therapy, targeted therapy, or radiotherapy; 3. Patients who underwent mastectomy and only had sentinel lymph node biopsy; 4. History of contralateral breast cancer or other second primary malignant tumor (excluding basal cell carcinoma of the skin and cervical carcinoma in situ); 5. Previous history of chest radiotherapy; 6. Presence of severe heart, lung, liver, kidney, hematopoietic system, or nervous system diseases, or mental disorders; 7. Presence of scleroderma or active systemic lupus erythematosus or other autoimmune diseases; 8. Pregnant and breastfeeding patients.

Table 2. The specific definitions of the study endpoints

IDFS	The time from the day the subject is randomized to the earliest occurrence of invasive cancer local recurrence, distant metastasis, or death, but does not include contralateral breast second primary cancer.
AFR	Any ipsilateral chest wall, breast, regional lymph node recurrence, or distant metastasis event that occurs during the follow-up period.
LRFS	The time from the day the subject is randomized to the earliest occurrence of ipsilateral chest wall, breast, or regional lymph node recurrence or death.
DMFS	The time from the day the subject is randomized to the earliest occurrence of distant metastasis or death.
RFS	The time from the day the subject is randomized to the earliest occurrence of ipsilateral chest wall, breast, regional lymph node recurrence, distant metastasis, or death.
OS	The time from the day the subject is randomized until the patient's death.
DFS	The time from the day the subject is randomized to the recurrence of the disease or the patient's death due to disease progression.
BCSM	The time from the day the subject is randomized to death from breast cancer.

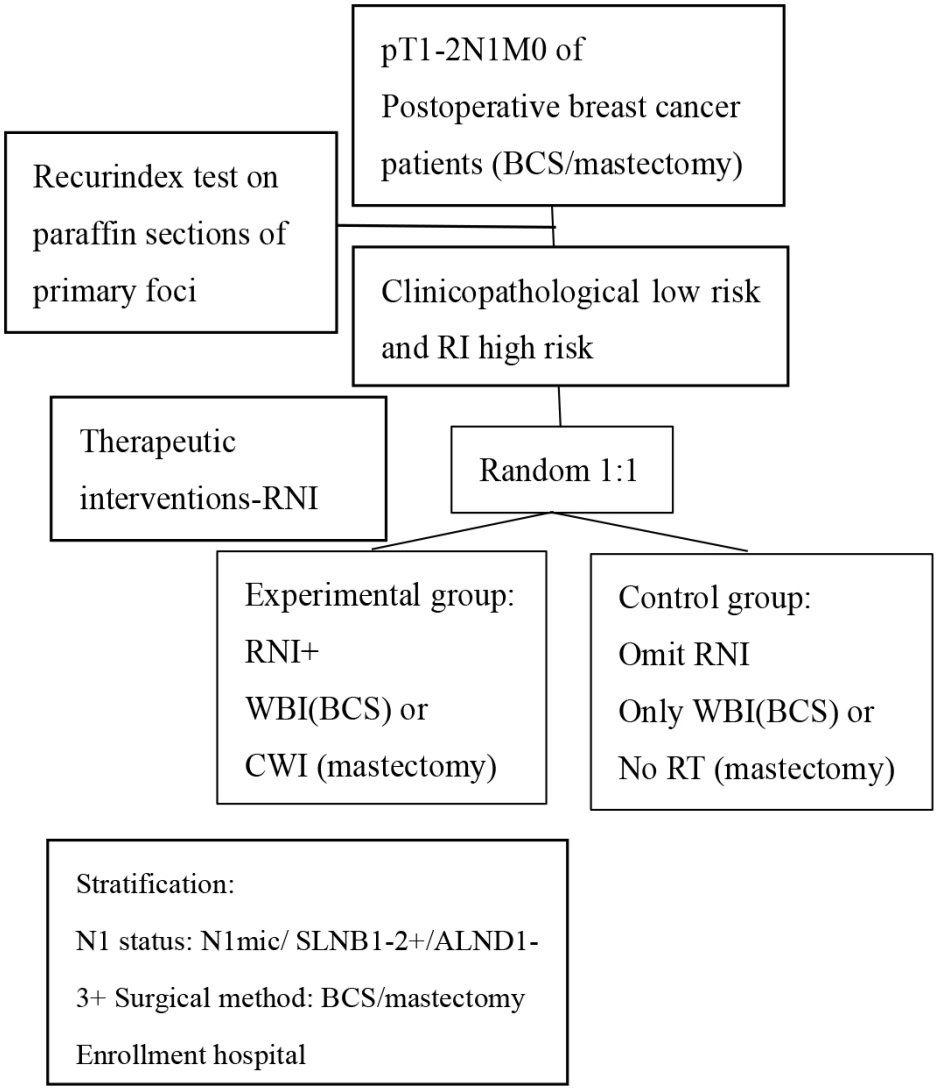

Figure 1 Research Process
Abbreviation: BCS, Breast conservative surgery; RI, Recurrence Index; RNI, Regional lymph node irradiation; WBI, Whole breast irradiation; CWI, Chest wall irradiation; RT, Radiotherapy; SLNB, Sentinel lymph node biopsy; ALND, Axillary lymph node dissection

90x114mm (305 x 305 DPI)

Supplementary 1. Trial registration data

Data category	Information
Primary registry and trial identifying number	Line 44 page 4
Date of registration in primary registry	April 1. 2023
Source(s) of monetary or material support	Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
Primary sponsor	Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
Secondary sponsor(s)	the Jiangsu Simcere Pharmaceutical Co., Ltd., Jiangsu Simcere Diagnostics Co., Ltd
Contact for public queries	Xiaobo Huang, MD. [huangxbo@mail.sysu.edu.cn]
Contact for scientific queries	Xiaobo Huang, MD. Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China
Public title	**RIGAIN Study**
Scientific title	Line 3 page 3
Countries of recruitment	Line 29 page 4
Health condition(s) or problem(s) studied	Regional lymph node irradiation
Intervention(s)	Line 26 page 4
Key inclusion and exclusion criteria	*Table 1* Line 3 page 18
Study type	Line 27 page 4
Date of first enrolment	Line 38 page 4
Target sample size	Line 33 page 4
Recruitment status	Recruiting
Primary outcome(s)	Line 19 page 8
Key secondary outcomes	Line 19 page 8

Supplementary 2. Quality of Life Questionnaire EORTC QLQ-C30 (version 3)

We are interested in learning some information about you and your health status. Please answer all of the following questions independently and circle the answer that is most appropriate for you. There are no "correct" or "incorrect" answers. The information you provide will be kept strictly confidential.

Please fill in your last name: _____

Date of birth (year, month, day): _____

Today's date (year, month, day): _____

	No	A little	Some	Very much
1.Do you feel difficulty when you do some laborious movements, such as lifting heavy shopping bags or luggage?	1	2	3	4
2. Do you find it difficult to walk long distances?	1	2	3	4
3. Do you find it difficult to walk short distances outdoors?	1	2	3	4
4. During the day, do you have to lie in bed or sit in a chair?	1	2	3	4
5. Do you need assistance with eating, dressing, washing or going to the bathroom?	1	2	3	4
In the past week:	1	2	3	4
6. Are your work or daily activities limited by physical ability?	1	2	3	4
7. Are your hobbies and leisure activities physically limited?	1	2	3	4
8. Do you ever feel short of breath?	1	2	3	4
9. Have you ever had any pain?	1	2	3	4
10. Have you ever needed rest?	1	2	3	4
11. Have you ever felt sleep deprived?	1	2	3	4
12. Have you ever felt weak?	1	2	3	4
13. Have you ever felt a lack of appetite?	1	2	3	4
14. Have you ever felt nauseous and wanted to vomit?	1	2	3	4
15. Have you ever vomited?	1	2	3	4
16. Have you ever had constipation?	1	2	3	4
17. Have you ever had diarrhea?	1	2	3	4
18. Do you ever feel tired?	1	2	3	4
19. Does pain interfere with your daily activities?	1	2	3	4
20. Do you have difficulty concentrating on things, such as reading the newspaper or watching TV?	1	2	3	4
21. Do you ever feel nervous?	1	2	3	4
22. Do you ever feel worried?	1	2	3	4
23. Do you ever feel easily irritated?	1	2	3	4
24. Do you ever feel depressed?	1	2	3	4
25. Do you ever have trouble remembering things?	1	2	3	4
26. Has your medical condition or treatment process interfered with your family life?	1	2	3	4
27. Has your medical condition or treatment interfered with your social activities?	1	2	3	4
28. Has your medical condition or treatment process caused you financial difficulties?	1	2	3	4

For the following questions, the numbers 1-7 represent a scale from "very poor" to "very good".

29. How would you rate your overall health in the past week?

1	2	3	4	5	6	7
very poor" to						very good

30. How would you rate the overall quality of your life in the past week?

1	2	3	4	5	6	7
very poor" to						very good

Patients sometimes have the following clinical symptoms. Please indicate the extent of these clinical symptoms or problems you have had in the past week, circling the answer that best applies to you.

	No	A little	Some	Very much
31. Do you cough a lot?	1	2	3	4
32. Do you cough up blood (blood in sputum)?	1	2	3	4
33. Do you feel short of breath when you rest?	1	2	3	4
34. Do you feel short of breath when you take a walk?	1	2	3	4
35. Do you feel short of breath when climbing stairs?	1	2	3	4
36. Have you ever had pain in your mouth or tongue?	1	2	3	4
37. Have you ever had difficulty swallowing?	1	2	3	4
38. Have you ever had tingling/numbness in your hands and feet?	1	2	3	4
39. Have you ever had hair loss?	1	2	3	4
40. Have you ever had chest pains?	1	2	3	4
41. Have you ever had pain in your arms or shoulders?	1	2	3	4
42. Have you ever had any pain in other parts of your body?	1	2	3	4
If yes, please write down the area:				
43. Have you ever taken any painkillers?				
1.Yes 2.No				
If you have used it, does it help much with pain?	1	2	3	4

Supplementary 3. Breast Cancer Survival Quality Scale EORTC QLQ-BR23

Please recall if you have experienced any of these symptoms or the extent of the problem and tick the appropriate number"√"

In the past 1 week	No	A little	More	A lot
1. Do you have dry mouth?	1	2	3	4
2. Do your food and drinks taste different than usual?	1	2	3	4
3. Do your eyes hurt, feel uncomfortable, or tear up?	1	2	3	4
4. Do you have hair loss?	1	2	3	4
5. If you have hair loss, does it bother you?	1	2	3	4
6. Do you feel sick or uncomfortable?	1	2	3	4
7. Is your face red and hot?	1	2	3	4
8. Do you have a headache?	1	2	3	4
9. Do you feel less physically attractive due to illness or treatment?	1	2	3	4
10. Do you feel less attractive as a woman due to illness or treatment?	1	2	3	4
11. Do you have difficulty looking at your naked body?	1	2	3	4
12. Are you dissatisfied with your body?	1	2	3	4
13. Are you worried about your future health?	1	2	3	4

In the past 4 week				
14. How interested are you in sex?	1	2	3	4
15. How active are you sexually (do you have sex often)? (With or without sex?)	1	2	3	4
16. If you have sex, to what extent does it bring you pleasure?	1	2	3	4

In the past 1 week
17. Do you have pain in your arm or shoulder?
18. Is your arm or hand swollen?
19. Do you have difficulty lifting or moving your arm to the side?
20. Do you have pain in the area of your affected breast?
21. Is the area of your affected breast swollen?
22. Do you have hypersensitivity in the affected breast area?
23.Do you have skin problems (e.g. itching, dryness, flaking) in the affected breast area?

Supplementary 4. Evaluation criteria for common adverse events (CTCAE Version 4.03)

(excerpt, normal common adverse event evaluation criteria is grade 0)

Adverse Events	Grading				
	1	2	3	4	5
Hemoglobin g/L	Normal value -10.0	10.0-8.0	8.0-6.5	＜6.5	
Leukocytes(10^9/L)	Normal value-3.0	3.0-2.0	2.0-1.0	＜1.0	
Neutrophils(10^9/L)	Normal value-1.5	1.5-1.0	1.0-0.5	＜0.5	
Platelets(10^9/L)	Normal value-75	75-50	50-25	＜25	
Transaminase ALT/AST	≤2.5×N	2.6-5.0×N	5.1-20×N	＞20×N	
Alkaline phosphatase	≤2.5×N	2.6-5.0×N	5.1-20×N	＞20×N	
Bilirubin	ULN-1.5×N	1.5-3.0×N	3.0-10×N	＞10×N	
Creatinine Cr	ULN-1.5×N	1.5-3.0×N	3.0-10×N	＞10×N	
Weight gain/loss	5.0-10%	10-20%	≥20%		
Vomiting	Vomiting 1 time in 24h during treatment	Vomiting 2-5 times in 24h during treatment	Vomiting ≥ 6 times in 24h during treatment or requiring fluids	Life-threatening and requires urgent treatment	Death
Coughing sputum	Occasional/mild coughing of sputum	Moderate cough and sputum; interferes with instrumental daily life	Persistent heavy coughing and limited personal self-care		
Pneumonia	Asymptomatic; clinical examination or diagnostic findings only; no intervention required	Symptomatic (mild cough and/or dyspnea, with or without fever); requires clinical intervention; interferes with instrumental daily	Severe symptoms; limited personal autonomy; need for oxygen	Life-threatening respiratory dysfunction; requiring urgent treatment (tracheotomy or intubation)	Death

		life			
Acute coronary syndrome		Symptomatic, progressive angina; normal cardiac enzymes; hemodynamically stable	Symptomatic, unstable angina with/ or acute myocardial infarction, abnormal cardiac enzymatic parameters, hemodynamically stable	Symptomatic, unstable angina with/ or acute myocardial infarction, abnormal cardiac enzymatic parameters, hemodynamic instability	Death
Left ventricular systolic insufficiency			Symptoms of decreased ejection fraction	Uncontrollable heart failure with declining ejection fraction requiring urgent intervention	Death
Heart Failure	Asymptomatic, with abnormalities detected by laboratory tests (e.g., natriuretic peptide) or cardiac imaging	Mild to moderate symptoms with activity or exercise	Symptoms occur at rest or with light activity or exercise; requires treatment	Life-threatening; requires urgent treatment (e.g. continuous infusion therapy or mechanically assisted circulation)	Death
Limb edema	Comparison using the greatest difference in volume or circumference, with 5% to 10% variation between limbs; edema or blurred anatomy that can only be detected on close examination	Comparison using the largest difference in volume or circumference, 10% <~30% difference between limbs; disappearance of skin folds; apparent loss of limb anatomy, change in	>30% volume variation between limbs; severe changes in limb shape; limited personal autonomy		

		shape; interferes with instrumental daily living			
Neurotoxicity - Sensory	Mild sensory abnormalities (including paresthesia), absence of deep tendon reflexes	Moderate objective sensory deficit or sensory abnormalities (including tingling)	Severe objective sensory loss or sensory abnormalities that affect daily life	Persistent sensory loss, affecting function	Death
Neurotoxicity-motor	Self-perceived weakness with no objective findings	Moderate self-conscious weakness; no significant functional impairment	Self-perceived weakness with functional impairment	Paralysis	Death

ULN, Upper limit of normal value

Supplementary 5. Scoring criteria for late radiation injury (RTOG/EORTC 1995)

Organ Tissue	Grading				
	0	1	2	3	4
Skin	No change	Mild atrophy, hyperpigmentation, partial hair loss	Lamellar atrophy, moderate capillary dilatation, total hair loss	Significa ophy, marked c ary dilation	Ulcers
Subcutaneous tissue	No change	Mild sclerosis (fibrosis) and loss of subcutaneous adipose tissue	Moderate fibrosis but asymptomatic, slight constriction of irradiated field <10% of the side length	Severe s is and loss of subcu us tissue. Constric of the irradiated >10% border le g	Necrosis
Lungs	No change	Asymptomatic or mildly symptomatic (dry cough), mild imaging signs	Moderate symptomatic pulmonary fibrosis or pneumonia (severe cough), hypothermia, patchy imaging	Severe s mptomatic pulmonary fibrosis or pneumonia w th dense imaging, ha es	severe respiratory insufficiency requiring continuous oxygenation or assisted ventilation
Heart	No change	Asymptomatic or mildly symptomatic; temporary T-wave inversion and ST changes; sinus tachycardia >110 beats/min at rest	Moderate exertional angina; mild pericarditis; normal heart size; persistent T-wave abnormalities and ST changes; low QRS waves	Severe angina pectoris; pericardial effusion; constrictive pericarditis; moderate heart failure; cardiac enlargement; abnorma electrocardiogram	pericardial tamponade; severe heart failure; severe constrictive pericarditis

Supplementary 6. Harris Cosmetic Grade Rating of Cosmetic Breast Preservation/Reconstructive Surgery Results

Level	Double breast symmetry	Double nipple level gap	Breast shape on the affected side	Skin
Excellent, Good	Symmetries	≤2cm	No significant difference with the healthy side, normal appearance, no deformation of the breast lift due to scarring, no difference between the affected side and the healthy side in feel	Normal
General	Symmetries	2cm-3cm	The shape of the affected breast is basically normal or slightly smaller than the healthy side, and the feel of the affected side is slightly worse.	Lightened or shiny color
Bad	Obvious asymmetry	＞3cm	The appearance of the affected side of the breast changes and is significantly smaller than the healthy side, and feels poorly in the hand	Thick, rubber-like, rough

Supplementary 7. Baker's classification of the prosthetic envelope

Grading	Breast Implants
I (no accessible envelope)	Breast implants feel as soft as non-operated breasts
II(Lightly hardened)	The softness of the breast is slightly worse, the implant can be touched but not seen
III(Heavy hardening)	Harder breasts, implants can be easily touched out or visible deformation of the implant
IV(severe contracture)	Breasts are hard, painful when touched, skin temperature becomes cold, deformation is obvious

Only Baker grade III and IV are defined as periosteal contracture and require reoperation

Supplementary 8. Radiotherapy-related adverse reactions and their treatment

1 Radiation-induced skin damage

Early skin reactions are those that occur within three months after the start of radiotherapy and are the most common complications in breast radiotherapy. Approximately 92% of patients receiving post-lumpectomy radiotherapy will experience acute radiation-induced skin reactions, mostly grade 1 or 2 mild reactions, with a wet desquamation incidence rate of about 3%. Patients undergoing mastectomy will almost always experience acute radiation-induced skin reactions, mostly grade 2 reactions, with a wet desquamation incidence rate of about 10-20%.

Prevention is the main approach to managing radiation-induced skin complications. For grade 1 and 2 injuries, conservative treatment is primarily used. Patients should wear loose, cotton open-front underwear, avoid friction and pressure on the skin in the irradiation area, avoid using irritating products such as soap and shower gel, avoid bathing with hot water or showering the irradiation area, and not apply chemical ointments or adhesive tape. If the skin is red, swollen, itchy, or painful, do not scratch it or apply medication randomly. Follow the doctor's advice for medication, such as triethanolamine cream, compound vitamin B12 solution, and medical radiation protectants. Wet dermatitis can be treated with exposure therapy, keeping the area dry and avoiding secondary infections. Wet dermatitis that does not heal after more than two months may develop into skin necrosis, often requiring surgical treatment, with skin grafting for larger areas.

Late skin reactions include local hyperpigmentation, telangiectasia, atrophy, and fibrosis. For chronic radiation dermatitis with recurrent ulceration and significant worsening, surgery is often used to prevent malignant transformation.

2 Pharyngeal and esophageal reactions

Irradiation of the supraclavicular area can cause pharyngeal pain and difficulty swallowing, which are generally mild and self-limiting. Prevention methods include using new radiotherapy techniques, accurate positioning, precise delineation of the target area, rational design of radiation fields, and reducing or avoiding irradiation of organs at risk. Symptomatic support treatment is provided for severe reactions.

3 Radiation-induced lung injury

Radiation-induced lung injury includes early radiation pneumonitis occurring within 3 months after radiotherapy and late radiation-induced pulmonary fibrosis occurring after 3 months. Approximately 2/3 of patients will develop asymptomatic radiation pneumonitis, which does not require treatment. The incidence of symptomatic radiation pneumonitis is between 1% and 5%, usually occurring within 2 months after radiotherapy or within 6 months after radiotherapy. Patients have symptoms and signs of pneumonia, which can manifest as cough, sputum, or fever, and in severe cases, dyspnea and hypoxia. In particular, when imaging examinations (chest X-rays and CT scans) show inflammatory exudative changes in the lung tissue within the irradiation field, symptomatic radiation pneumonitis can be diagnosed after excluding lung metastasis and tuberculosis. Supportive treatment, including hormones, oxygen therapy, and even mechanical ventilation, can provide complete relief, but some patients may still develop pulmonary fibrosis within 6-12 months, even with treatment. Pulmonary fibrosis is a late injury caused by damage to the lung interstitium and pleura, and in severe cases, it can be life-threatening.

There is currently no specific treatment for radiation pneumonitis, so prevention is more important than treatment. For patients undergoing whole-breast irradiation alone, it is recommended to use a dose-volume constraint of V20 < 22% for the ipsilateral lung. For those receiving irradiation of the supraclavicular lymph node region, a dose-volume constraint of V20 < 34% and V30 < 22% should be used for the ipsilateral lung to further evaluate the overall radiotherapy plan.

4 Radiation-induced heart damage

Radiation-induced heart disease (RIHD) initially manifests as acute pericarditis and later as coronary artery disease, chronic pericarditis, myocardial fibrosis, cardiomyopathy, heart valve damage, and cardiac conduction abnormalities. A 2013 New England Journal article reported that for every 1 Gy increase in the average dose to the heart, the incidence of major coronary events increased by 7.4%.

Reducing the risk of RIHD is also focused on prevention. The most fundamental measure is to minimize or avoid radiation exposure to the heart during radiotherapy. The Chinese Anti-Cancer Association Breast Cancer Diagnosis and Treatment Guidelines and Standards (2015 Edition) recommend that the average radiation dose to the heart should be assessed to be at least below 8 Gy. It is recommended to limit the heart's V30 to less than 10%. In addition, for high-risk populations of RIHD or those with cardiovascular disease, drugs that have a protective effect on the cardiovascular system should be used as soon as possible. Regular cardiac ultrasound follow-ups should be conducted during the follow-up phase.

5 Upper limb edema

Edema in the affected upper limb is one of the common complications after breast cancer surgery and/or radiotherapy, and the extent of surgery is an important influencing factor. AMAROS research reported that the 1-year, 3-year, and 5-year lymphedema incidence rates for the ALND group were 28%, 23%, and 23%, respectively, significantly higher than the 15%, 14%, and 11% for the SLNB + axillary radiotherapy group. The incidence of upper limb lymphedema after axillary lymph node biopsy alone is 5%. Edema caused by radiotherapy usually occurs 1 to 2 months after the end of radiotherapy. Depending on the time of onset, upper limb edema can be divided into early edema and delayed edema occurring after several years. Upper limb edema caused by tumor recurrence in the axilla and supraclavicular region is not considered a true post-treatment complication.

The main prevention method for upper limb edema is to reduce axillary dissection, and postoperative progressive functional exercise is the key to preventing upper limb edema. When upper limb edema occurs, manual massage or compression therapy can be used.

6 Brachial plexus nerve injury

Radiation-induced brachial plexus nerve injury is a rare late complication after breast cancer radiotherapy, with an incidence rate of 1%-4%. Early symptoms include sensory and motor disorders in the affected limb and pain, often accompanied by severe nocturnal pain. Some cases may also have lymphedema, with progressively worsening functional impairment. In the late stage, this can lead to the loss of function of the entire limb, causing lifelong disability for the patient and severely affecting the patient's daily life and rest, with a significant impact on their mental health and quality of life. A preliminary diagnosis can be made based on the patient's radiotherapy history, asymptomatic intervals, and clinical features in clinical practice. However, it is necessary to rule out brachial plexus nerve injury caused by tumor metastasis or compression.

Radiation-induced brachial plexus nerve injury is irreversible, and there is currently no ideal treatment

method, so prevention is crucial. It is essential to strictly follow the indications for radiotherapy in the lymphatic drainage area and pay attention to the radiotherapy range and radiation dose. For cases without severe pain, active measures should be taken to improve the blood supply of the nerves and surrounding soft tissues, and the earlier the diagnosis and treatment, the better the results. For advanced cases, treatment is aimed at relieving pain and improving quality of life.

7 Second primary tumors

Second primary tumors that can occur after breast cancer treatment include contralateral breast cancer and other malignant tumors such as lung cancer and soft tissue sarcomas. If these second primary tumors can be diagnosed and treated early, they do not affect the patient's survival. Therefore, regular follow-up of patients should be strengthened in clinical practice.

8 Rib fractures

The incidence is less than 1%. In most cases, patients have no noticeable symptoms, and fractures are discovered during bone scans or X-ray examinations. A small number of patients may experience chest wall or rib pain, which generally heals on its own without the need for special treatment.

9 Other side effects

During radiotherapy, patients may experience mild loss of appetite and fatigue. Therefore, it is important to adjust the diet reasonably, advocating for a "high protein, high vitamin, low fat" diet to maintain a balanced nutrition. Regularly review routine blood tests, and if a decrease in white blood cells is found, there is a risk of infection. In such cases, it may be necessary to temporarily pause radiotherapy and follow the doctor's advice for symptomatic supportive treatment.

Supplementary 9. Radiation Target Volume Naming and Delineation

I. Purpose:

To ensure the smooth conduct of the clinical trial and to guarantee the quality of the clinical trial.

II. Scope:

This clinical trial.

III. Procedures:

一、General Principles of Target Delineation: To be performed on plain CT scans.

二、RNI + WBI (BCS)/CWI (Mastectomy)

Standards	**2.1 Whole Breast Target CTV_2**
Superior Boundary	Upper edge of the palpable/CT-visible gland.
Inferior	Lower edge of the palpable/CT-visible gland.
Anterior	5 mm beneath the skin; for small and thin breasts, adjust the anterior boundary to 0.3 cm beneath the skin or even closer.
Posterior	1-2 mm behind the surface of the pectoralis major fascia (adjacent to the retromammary space), leaving no fat gap, including the lymph nodes between the pectoralis major and minor muscles and unsampled axillary levels I and II, excluding ribs/intercostal muscles.
Medial	Parasternum, at least to the medial edge of the internal mammary vessels.
Lateral	Lateral edge of the palpable/CT-visible gland, anterior to the thoracodorsal artery, and anterior edge of the latissimus dorsi muscle.

Standards	**2.2Tumor Bed and CTV_1**
Tumor Bed	The boundaries of the tumor bed are determined by: The positions of the surgical clips; it is recommended to place clips at five points: left, right, superior, inferior, and posterior.The extent of seroma, ensuring that any seroma within the gland and beneath the scar is included.
CTV_1	Includes the breast glandular tissue and soft tissue extending 10-15 mm beyond the surgical tumor bed. For patients who underwent segmental resection, a smaller margin of around 10 mm is recommended. If there is no glandular tissue beyond the tumor bed, the margin can be appropriately reduced. For patients with positive margins, extensive intraductal component (EIC), or severe atypical ductal hyperplasia (ADH), the margin must be appropriately expanded.

Standards	**2.3 Integrated Target Volume CTV_2 for Whole Breast and Low/Mid Axillary Regions**		
Whole Breast	Refer to the Whole Breast Target CTV_2		
Axilla	Axillary Level I: Anatomically marked by the lateral edge of the pectoralis minor.		
	Axillary Level I	Axillary Level II	Rotter's Lymph Nodes
Superior	Where the axillary vessels cross the lateral edge of the pectoralis minor	Where the axillary vessels cross the medial edge of the pectoralis minor	Includes the superior side of the axillary artery and 5 mm above the axillary vein
Inferior	Where the pectoralis major inserts into the ribs	Where the axillary vessels cross the lateral edge of the pectoralis minor	Inferior boundary of Axillary Level II
Anterior	Anterior surface of the pectoralis major and latissimus dorsi	Anterior surface of the pectoralis minor	Posterior surface of the pectoralis major
Posterior	Anterior surface of the subscapularis muscle	Ribs and intercostal muscles	Anterior surface of the pectoralis minor
Medial	Lateral edge of the pectoralis minor	Medial edge of the pectoralis minor	Medial edge of the pectoralis minor
Lateral	Medial surface of the latissimus dorsi	Lateral edge of the pectoralis minor	Lateral edge of the pectoralis minor

Standards	**Chest Wall Target CTV_CW**
Superior	Clinical markers/subclavian head 0.5-1 cm
Inferior	Clinical markers/inferior edge of the contralateral breast fold
Anterior	Skin, excluding the wire
Posterior	Ribs and intercostal muscles
Medial	Clinical markers/junction of the sternum and ribs
Lateral	Clinical markers/thoracodorsal vessels and the anterior edge of the latissimus dorsi muscle
Note: 1. The entire scar should be included, and the target area should not be reduced within 2 cm above and below the scar. 2. Postoperative changes visible on CT (such as granulomas, fibrosis, and spiculated muscle	

irritation signs) should be included.

Standards	2.5 Supraclavicular and Infraclavicular Lymph Node Area CTV_LN
Superior	Inferior edge of the cricoid cartilage
Inferior	0.5-1 cm below the clavicular head, at the level where the brachiocephalic vein disappears, merging with the whole breast/chest wall target area
Anterior	Superior part: posterior surface of the sternocleidomastoid muscle; Inferior part: posterior surface of the pectoralis major muscle
Posterior	Superior part: posterior edge of the anterior scalene muscle; Inferior part: anterior edge of the ribs and intercostal muscles
Medial	Superior part: internal jugular vein, covering the interscalene triangle to the level of the transverse cervical artery and vein; Inferior part: junction of the subclavian vein and internal jugular vein
Lateral	Superior part: lateral edge of the sternocleidomastoid muscle; Inferior part: lateral edge of the pectoralis minor muscle
Note: 1. Avoid the surgically treated axillary area (Level I and part of Level II). 2. Include the non-surgically treated area of axillary Level II.	

Standards	2.6 Internal Mammary Lymph Node Area CTV_IMN
Superior	Injection into the internal area of the clavicle; for high-risk patients, extend to the junction of the internal jugular vein, subclavian vein, or brachiocephalic vein, and the internal mammary vein
Inferior	Upper edge of the fourth rib cartilage
Anterior	Posterior surface of the pectoralis major muscle and the posterior surface of the sternum
Posterior	Pleura or 5 mm behind the posterior aspect of the internal mammary vessels
Medial	5 mm inside the internal mammary vessels, covering the space between the sternum and the vessels
Lateral	5 mm outside the internal mammary vessels, to the outer edge of the brachiocephalic vein
Note: 1. For high-risk patients, the superior boundary extends to the junction of the internal jugular vein, subclavian vein, or brachiocephalic vein, and the internal mammary vein. 2. It is recommended to extend the coverage in the medial and lateral directions (at least) by 5 mm.	

Standards	2.7 Intraclavicular Lymph Node CTV_intraclavicular-LN
Superior	Level of the transverse cervical artery
Inferior	Upper edge of the brachiocephalic trunk
Medial	Midline of the body
Lateral	Inner boundary of the upper clavicle region

Note:

1. When irradiating the internal mammary lymph nodes, routine delineation is recommended.
2. When there is capsular invasion of the lymph nodes in the axillary Level II/III region, routine delineation is recommended.
3. Patients with primary tumor invasion of the deep fascia or tumors located medially and superiorly within the breast may be considered for delineation.

三、Omission of RNI, WBI (BCS) only, no CWI (total mastectomy)

Standard	3.1 Whole Breast Target CTV_2
Superior	Upper edge of palpable/CT-visible gland.
Inferior	Lower edge of palpable/CT-visible gland.
Anterior	Subcutaneous tissue 5 mm beneath the skin; for thin/small breasts, adjust anterior boundary to 0.3 cm beneath the skin or even closer.
Posterior	1-2 mm behind the surface of the pectoralis major fascia (adjacent to the retromammary space), leaving no fat gap, excluding lymph nodes between pectoralis major and minor muscles and unsampled axillary levels I and II, excluding ribs/intercostal muscles.
Medial	Parasternal, at least to the medial edge of the internal mammary vessels.
Lateral	Lateral edge of palpable/CT-visible gland, anterior to the thoracodorsal artery, and anterior edge of the latissimus dorsi muscle.

Standard	3.2 Tumor Bed and CTV_1
Refer to Standard 2.2 Tumor Bed and CTV_1	

Standard	3.3 Integrated Target Volume CTV_2 for Whole Breast and Low/Mid Axillary
Refer to Standard 2.3 Tumor Bed and CTV_1	

Supplementary 10. Prescribed Radiation Dose and Evaluation

I. Purpose: To ensure the smooth conduct of the clinical trial and to guarantee the quality of the clinical trial.

II. Scope: This clinical trial.

III. Procedures:

1. **Prescribed Dose and Fractionation:**

   1.1. RNI + WBI (BCS)/CWI (mastectomy): 5000 cGy in 25 fractions, 200 cGy per day, five days a week. For breast-conserving patients, a boost to the tumor bed is required, sequentially administered after whole-breast irradiation, with a dose of 1000 cGy-1600 cGy in 5-8 fractions, 200 cGy per day. Alternatively, a hypofractionated radiotherapy scheme can be chosen, with a radiation dose of 4000-4256 cGy in 15 to 16 fractions. For breast-conserving patients, a sequential tumor bed boost is performed after completion of whole breast irradiation, as determined by individual center investigators. It can be delivered using conventional fractionation, with a dose of 1000 cGy in 5 fractions at a rate of 200 cGy per day, or by using hypofractionation, with a dose of 798 cGy-1064 cGy in 3 to 4 fractions at a rate of 266 cGy per day. If there are high-risk factors for local recurrence, such as positive surgical margins, close margins, or young age, the radiation dose for the tumor bed boost may be increased to 1400-1600 cGy in 7 to 8 fractions at a rate of 200 cGy per day.

   1.2. RNI omitted, only WBI (BCS), no CWI (mastectomy): 4000 cGy in 15 fractions or 4250 cGy in 16 fractions, 266 cGy per day (hypofractionation), five days a week; or 5000 cGy in 25 fractions, 200 cGy per day (conventional fractionation), five days a week. A boost to the tumor bed is required, sequentially administered after whole-breast irradiation, with a dose of 1000 cGy-1600 cGy in 5-8 fractions, 200 cGy per day.

2. **Dose Distribution and Organs at Risk Limits:**

   2.1. The prescribed dose should cover at least 95% of the PTV in 95% of the target area, with specific dose distribution determined by each center's policy. 2.2. Organs at risk limits should refer to the Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC) standards.

   2.3. For patients with left breast cancer, RNI + WBI (for BCS) / CWI (for mastectomy)

should have a mean heart dose (Dmean) $\leqslant$ 8 Gy, while WBI (for BCS) alone should be limited to a Dmean $\leqslant$ 5 Gy.

Supplementary **11** Dose distribution and organ endangerment limits

Target Volume	Dmax	Dmin
Whole Breast PTV_2	≤107%	≥90%
Tumor Bed PTV_1	≤107%	≥90%
Whole Breast and Low-to-Mid Axilla Integrated Target Volume PTV_2	≤107%	≥90%
Chest Wall PTV_CW	≤110%	≥90%
Supraclavicular (±intranodal clavicular) PTV_LN	≤110%	≥90%
Internal Mammary PTV_IMN	≤110%	≥80%

Supplementary 12. Organ dose/volume/impact data for routine split exposures (except where noted): QUANTEC

Organs	Volume	Type of irradiation (partial organ or specially indicated)	Observation index	Dose (Gy) or dose volume parameter	Incidence (%)	Dose volume parameter description
Spinal Cord	Partial spinal cord Thoracic medulla	3DCRT	Spinal cord lesions	Dmax=50	0.02	Includes all spinal cord cross-sections
Pharynx	pharyngeal constrictor muscle	3DCRT	Dysphagia and shortness of breath	Dmean＜50	＜20	
larynx	Total larynx	3DCRT	Edema	Dmean＜44	＜20	No chemotherapy, based on a single study of patients without laryngeal cancer
	Total larynx	3DCRT	Edema	V50＜27%	＜20	
lung	whole lung	3DCRT	Pneumonia	V20≤30%	＜20	Double lung. Slow dose response Without whole lung treatment irradiation
	whole lung	3DCRT	Pneumonia	Dmean=7	5	
	whole lung	3DCRT	Pneumonia	Dmean=13	10	
	whole lung	3DCRT	Pneumonia	Dmean=20	20	
	whole lung	3DCRT	Pneumonia	Dmean=24	30	
	whole lung	3DCRT	Pneumonia	Dmean=27	40	
Esophagus	Whole Esophagus	3DCRT	≥3 Grade acute esophagitis	Dmean＜34	5-20	Contains various dose limiting factors. Seems to be related to dose volume
	Whole Esophagus	3DCRT	≥ grade 2 acute esophagitis	V35＜50%	＜30	
	Whole Esophagus	3DCRT	≥ grade 2 acute esophagitis	V50＜40%	＜30	
heart	Pericardium	3DCRT	pericarditis	Dmean＜26	＜15	Based on individual studies
	Pericardium	3DCRT	pericarditis	V30＜46%	＜15	
	Whole heart	3DCRT	distant cardiac	V25＜46%	＜1	High standards for

			death			assessing security based on predictive models
Liver	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 30-32	＜5	Exclude patients with existing liver disease or liver cancer
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 42	＜50	Patients with liver disease or hepatocellular carcinoma with a Child-Pugh rating of A, but not active hepatitis B, were included as observation indicators
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 28	＜5	
	Whole Liver - GTV	3DCRT	Typical RILD	Dmean＜ 36	＜50	
	Whole stomach	3DCRT	Ulcer	D100＜45	＜7	

QUANTEC: Quantitative Analysis of Illumination Response in Clinically Normal Tissues; 3DCRT: Three-Dimensional Conformal Radiotherapy; GTV: Gross Tumor Volume; RILD: Radioactive Liver Injury; RTOG: Radiation Therapy Oncology Group of Amer

Supplementary 13. Research Schedule

Research Phase	Screening	Radiotherapy period				Follow up period										
Visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Time	-3M-0	1W	2W	3W	4-7W	6M	12M	18M	24M	30M	36M	42M	48M	54M	60M	In the event of a relapse
Informed consent	×															
Inclusion/exclusion criteria	×															
Frozen/paraffin tissue	×															
Pathology	×*															
Tumor markers	×					×	×	×	×	×	×	×	×	×	×	×
Breast + LN ultrasound/MR	×					×	×	×	×	×	×	×	×	×	×	
Mammography	×*						×		×		×		×		×	
Chest X-ray/CT	×					×	×	×	×	×	×	×	×	×	×	×
Abdominal ultrasound/CT	×					×	×	×	×	×	×	×	×	×	×	×
Thyroid + LN ultrasound	×					×	×	×	×	×	×	×	×	×	×	
Bone Scan	×*						×		×		×		×		×	×
Cranial CT/MRI																×***
Demographic features	×															
Medical history	×															
Physical examination	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	

Upper and lower arm circumference measurement◆	×				×		×		×		×		×		×	
Body weight	×				×		×		×		×		×		×	
ECOG score	×				×		×		×		×		×		×	
Blood count	×**	×	×	×	×	×	×	×	×	×	×		×	×	×	×
Liver and kidney function	×**				×	×	×	×	×	×	×		×	×	×	×
Infectious disease	×*															
Serum pregnancy test	×**															
EORTC QLQ-C30	×**				×		×		×		×		×		×	
EORTC QLQ-BR23	×**				×		×		×		×		×		×	
Harris	×**						×		×		×		×		×	
Baker	×**						×		×		×		×		×	
CTCAE V4.03	×**	×	×	×	×		×	×	×		×		×		×	
Acute radiation reaction		×	×	×	×											
Late radiation injury							×	×	×		×		×		×	

◆Upper and lower arm circumference measurements: measured at the bilateral metacarpophalangeal joints (mid-metacarpal), at the wrist, and at the distal 10 cm and proximal 15 cm of the elbow epicondyle.

* Within 12 months prior to randomization

** Within 14 days before randomization

*** When cranial symptoms were present

Supplementary 14. **Data Management**

1. Source Data Recording

Monitoring personnel will verify source data to confirm that the data entered by authorized personnel at the trial center into the electronic Case Report Form (eCRF), i.e., the source data, is correct, complete, and indeed originated from the source documents.

Source documents (paper or electronic) refer to patient data recorded at the earliest time and include but are not limited to: hospital records, laboratory records, memos, patient-reported outcomes, assessment checklists, data recorded on automated instruments, microfiche, photographic films, X-rays, etc.

Source documents requiring verification of data integrity and validity must not be altered or destroyed and must be retained in accordance with the applicable regulatory retention policies.

For source data verification, investigators and trial institutions must provide monitoring personnel with direct access to relevant source documents and reports for audit purposes and Institutional Ethics Board/Ethics Committee (IEB/EC) review. Trial centers must also allow regulatory authorities to conduct inspections.

2. Use of Computer Systems

When clinical observation results are entered directly into the computerized medical record system of the study center, electronic records may be considered as source documents if the system has been validated according to regulatory requirements for computerized systems adopted in clinical research. Original data should be saved using appropriate computerized data collection systems. If original data requires modification, the system must retain visual inspection audit trails showing the original data and reasons for modification, along with the names and dates of modification.

3. Case Report Forms

This study utilizes electronic Case Report Forms (e-CRFs) for data collection, completed using the Electronic Data Capture (EDC) system specified by the principal investigator. The designated vendor appointed by the principal investigator will provide training to trial centers and a suitable e-CRF completion manual to the research centers.

All e-CRFs are completed by designated, trained personnel at the trial center, and the investigator or designated personnel must review, electronically sign, and date the e-CRFs.

4. Data Quality Assurance

The clinical trial office where the principal investigator is located is responsible for data management for this study, including monitoring/audit of data quality. Clinical research data will be collected via eCRFs using the EDC system. Data entry into the ECD system will be the responsibility of the research center. In case of discrepancies, the clinical trial office where the principal investigator is located will request explanations from the research center, and this process will be electronically resolved within the EDC system.

The sponsor will develop an EDC study quality standards document outlining methods for quality checks on the data.

Data from center laboratories will be sent directly to the principal investigator, who will process and electronically transfer these data according to the standard operating procedures recognized by the

principal investigator for center laboratories.

e-CRFs and correction documents will be retained in the EDC system during the auditing process. The data retained by the principal investigator will be systematically backed up according to standard operating procedures recognized by the principal investigator for the vendor, and records of research data retention will be kept.

5. Independent Data Safety Committee

A Data Safety Committee will be established, consisting of an odd number of members (typically 5), who are independent of the project team and have signed confidentiality agreements. The committee will primarily conduct a review analysis of positive results (subject relapse and metastasis) and understand the actual study results (without statistical analysis) when half of the subjects are enrolled, voting on whether adjustments to the study protocol are necessary

Supplementary 15. **Quality Management Plan**

1 Quality Control

1) Qualifications of Study Personnel: All personnel involved in this trial, including investigators, nurses, statisticians, clinical trial observers, etc., must undergo clinical trial training and work under the guidance of senior professionals.
2) Investigators and other personnel involved in the study should fulfill their responsibilities and strictly adhere to the clinical trial protocol, employing standard operating procedures to ensure the implementation of quality control and quality assurance systems.
3) All observed results and findings in the clinical trial should be verified, and quality control must be conducted at every stage of data processing to ensure data integrity, accuracy, authenticity, and reliability.
4) Investigators and other personnel involved in the study should have sufficient time and reliable sources of subjects for conducting the study.
5) All projects involving imaging and laboratory testing should be carried out by units that comply with national standards.
6) Specimens requiring collection in this study should be collected by designated individuals, and follow-up data should be collected and stored by designated personnel.
7) Testing procedures should be conducted according to the specified Standard Operating Procedures (SOPs).
8) When modifications to the study protocol are required, the Ethics Committee should be convened according to SOPs, fully utilizing the Ethics Committee's functions to ensure the protection of subjects' interests.
9) Each participating unit should establish a file folder, save all original materials as required in the protocol, and arrange them in chronological order for verification purposes.
10) Contract research organizations must appoint trained monitors for the study, who should have relevant medical and pharmaceutical backgrounds, and conduct inspections of the research projects according to SOPs (including: pre-trial visits, initiation visits, routine monitoring visits, and end-of-study visits; see "Monitoring Plan").
11) Inspectors should systematically examine clinical trial-related activities and documents to assess whether the trial is conducted in accordance with the protocol, SOPs, and relevant regulatory requirements, and whether trial data are recorded in a timely, truthful, accurate, and complete manner. Inspections should be conducted by personnel not directly involved in the clinical trial.
12) The establishment of inspection work is to ensure that clinical trials are conducted in accordance with the requirements of the protocol, SOPs, and relevant regulations. Contents include: a) How is the clinical research operated? b) Is the implementation in line with the requirements of the study protocol? c) Is the principal investigator effectively and appropriately monitoring the progress of the study? d) How is the quality of the study: whether the study personnel, study centers, and data trial centers adhere to the requirements of SOPs? e) Are the data copied onto the Case Report Form (CRF) consistent with the original data? f) Overall trial quality (identifying the root causes of issues). g) Are study documents present? Are they stored systematically? Are they interpretable (can trial data be reconstructed from study documents)? h) Inspection of monitoring reports attempts to identify quality trends and consult on corrective measures for procedural issues that

have arisen.

13) Regular inspections, preparation of inspection reports, and holding meetings with relevant personnel to discuss issues identified during the inspection.

2 Monitoring Plan

The monitor conducts three types of visits: study initiation visits, routine visits, and close-out visits.

2.1 Study Initiation Visits

Meet with the principal investigator, establish a visit plan, and introduce the monitoring objectives and plans to the investigator. Review includes: training manuals, forms, study protocols, qualifications of participating researchers, and compliance with data management SOPs, etc. If necessary, a start-up meeting can be convened to discuss the protocol and work content with all doctors and other staff participating in the study, clarify each person's responsibilities, explain the SOP requirements for data entry standards, and the preservation of original data.

2.2 Routine Visits

① Before each visit, review the progress of the trial and unresolved issues from previous visits, contact the investigator to confirm the visit date, develop a plan and agenda for this visit, prepare the required documents and items for the visit.

② Meet with the investigator to explain the main tasks of this visit, understand the progress of the trial (subject enrollment status, CRF completion status, informed consent signing status, etc.), and the resolution of problems identified during previous visits.

③ Check and update the investigator's management files, verify the original documents and CRF forms (pay attention to compliance, completeness, consistency with the protocol, discovery, and reporting of SAEs), check trial materials (storage conditions, distribution and recovery records, compliance with protocol requirements).

④ Collect CRF forms.

⑤ Record any issues discovered, discuss and resolve the problems identified during this visit with the investigator, exchange progress and experiences with other research units.

⑥ Store items retrieved, signed informed consent forms, CRF forms, etc., as required.

⑦ Complete the visit report, update various records, track and resolve any issues discovered, and schedule follow-up visit plans.

⑧ SAEs that occur during the clinical trial must be reported to the Ethics Committee within 24 hours.

⑨ Any changes to the protocol, CRF forms, etc., during the trial require approval from the Ethics Committee. Documents to be submitted to the Ethics Committee during the trial include: protocol amendments, informed consent form amendments, SAE reports, recruitment advertisements (if used).

2.3 Close-Out Visits

① Review any outstanding issues from routine visits and confirm their resolution.

② Confirm the visit time, develop a plan and agenda for this visit.

③ Confirm the completeness and updating of the investigator's management files.

④ Confirm that all CRF forms have been collected.
⑤ Confirm the reporting and tracking of SAEs.
⑥ Check the records of the transport, distribution, and retrieval of various materials for the study.
⑦ Discuss and summarize, confirm any outstanding issues and follow-up work, explain the requirements for the preservation of trial-related documents.
⑧ Follow-up work: Complete the trial close-out monitoring visit report, notify the Ethics Committee of the trial's conclusion, continue to track and resolve any outstanding issues, and archive all documents. Documents to be submitted to the Ethics Committee after the trial ends include: trial closure letter, SAEs after trial closure.

3 Data Requirements

Protocol: After careful reading and agreement, the principal investigator must sign and strictly adhere to the protocol implementation.

Clinical Trial Data: All various original clinical trial data should be recorded promptly, truthfully, accurately, and completely, and copies of laboratory test reports should be retained. The principal investigator must retain records and documents of the study implementation process, including eCRFs, informed consent forms, laboratory test results, and radiotherapy plans, for 5 years after the completion or termination of the study, or for a longer period as required by regulatory authorities (whichever is longer). After this time period, the documents may be destroyed in accordance with local regulatory requirements.

4 Study Summary

Once the required total number of cases is reached and verified, the data analysis center performs data analysis. Based on the statistical analysis report, the responsible unit of the clinical trial and participating units write a summary of the clinical trial and sub-center summary table according to the principles of Good Clinical Practice (GCP) clinical trial guidance.

5 Research Funding

The RecurIndex-related testing expenses for this trial are provided by the collaborating party, with specific funding arrangements outlined in a signed contract.

6 Financial Transparency

The principal investigator is required to provide complete and accurate financial information in accordance with Chinese regulations, in order to submit comprehensive and accurate financial statements or disclosure statements to relevant health authorities. The principal investigator is responsible for providing financial information from the beginning to the completion of the study period.

Supplementary 16. Statistical Analysis

1. Population Analysis

Full Analysis Set (FAS): Efficacy analysis will be conducted for all cases randomized according to the intention-to-treat (ITT) principle, and analyzed based on their randomized groups, regardless of the actual radiation therapy group received.

Per-Protocol Set (PPS): Subjects from the FAS set will be excluded if they have major protocol violations that could potentially affect the primary efficacy endpoint IDFS analysis. Efficacy evaluation for this study will be conducted for both FAS and PPS sets, with FAS serving as the primary analysis set.

Safety Set (SS): All subjects randomized and receiving at least one session of radiation therapy belong to the safety analysis set. This dataset utilizes actual radiation therapy groups and is used for safety analysis.

2. Demographic and Baseline Characteristics

Descriptive statistical analysis will be conducted for demographic characteristics such as age, gender, height, weight, as well as other baseline features including medical history.

3. Participant Distribution

Descriptive statistical analysis will be performed on participant enrollment status, study completion status, premature study withdrawal, etc. A tabular summary will outline the distribution of participants across different analysis populations.

4. Efficacy Analysis

Efficacy analysis will be based on both FAS and PPS, with FAS serving as the primary analysis set.
Primary Endpoint Analysis: Invasive Disease-Free Survival (IDFS) serves as the primary efficacy endpoint of this study, defined as the time from randomization to the earliest occurrence of invasive cancer local recurrence, distant metastasis, or death, whichever comes first. The occurrence of invasive disease recurrence will be determined by the assessment results obtained by an independent review committee using pathological or imaging examinations. Patients who have not experienced invasive cancer local recurrence, distant metastasis, or death will have their last follow-up date considered as the censoring date. Patients who have not undergone imaging follow-up after baseline will have the randomization date considered as the censoring date. Cox proportional-hazards model will be used for the analysis of the primary endpoint to calculate the Hazard Ratio and its 95% confidence interval, adjusting for stratification factors and other covariates. Additionally, a Cox proportional-hazards model without covariates will be used to support the analysis results of the primary endpoint. Furthermore, the median invasive disease-free survival for both groups (Group A vs. Group B) will be calculated using the Kaplan-Meier (KM) method, including the 95% confidence interval. KM plots will be used to illustrate the trend of IDFS over time.
Secondary Endpoint Analysis: The analysis will compute the Annual Failure Rate (AFR) for each group, along with the corresponding 95% Clopper-Pearson confidence interval. Local recurrence-

free survival (LRFS), distant metastasis-free survival (DMFS), relapse-free survival (RFS), overall survival (OS), disease-free survival (DFS), and breast cancer-specific mortality (BCSM) will be analyzed using the KM method to determine the median values (including the 95% confidence interval). Changes in total scores of EORTC QLQ-C30 and EORTC QLQ-BS23 from baseline will also be summarized.

5. Safety Analysis

Safety analysis will be based on the Safety Set (SS).

The safety analysis will include all enrolled patients who have received at least one session of radiation therapy, grouped according to the actual treatment received by the patients.

Safety will be assessed by summarizing adverse events, changes in laboratory test results, vital sign changes, and exposure to study treatment, reported by treatment group.

All adverse events (AEs) will be classified according to the Medical Dictionary for Regulatory Activities (MedDRA) terminology and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC) Acute Radiation Morbidity Scoring Criteria (1995), and RTOG/EORTC Late Radiation Morbidity Scoring Criteria (1995). All adverse events occurring during treatment (Treatment Emergent Adverse Events, TEAEs, defined as events occurring within 30 days after the end of the last radiation therapy session), TEAEs of Grade 3 or higher, serious adverse events (SAEs), radiation-related SAEs, and TEAEs leading to trial discontinuation will be summarized by system organ class, preferred term, and group in terms of number and percentage. Additionally, the severity and relatedness of TEAEs to radiation therapy will also be summarized by system organ class, preferred term, and group. If a patient experiences the same adverse event multiple times, the maximum reported severity will be used for summarization.

6. Exploratory Studies

Exploratory studies will investigate the relationship between peripheral blood T lymphocyte subsets and the immunomodulatory effects of radiotherapy, as well as the relationship between circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and distant tumor eradication effects.

7. Interim Analysis

Interim analysis is not planned for this trial.

8. Final Analysis

The final analysis for this study is planned to be conducted when the last enrolled patient completes radiotherapy and reaches a follow-up of 5 years.

Supplementary 17. Physical status ECOG scoring criteria

ECOG scoring criteria	Scoring
Mobility is completely normal and does not differ in any way from that before the onset of the disease	0
Can walk freely and perform light physical activities, including general housework or office work, but cannot perform heavier physical activities	1
Able to walk freely and take care of themselves, but have lost the ability to work, and can get up and move around at least half of the time during the day	2
Only partially able to take care of themselves, bedridden or wheelchair bound for more than half of the day	3
Bedridden and unable to care for themselves	4
Death	5

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (*pg3. line 3*)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (*pg4. line 44*)
	2b	All items from the World Health Organization Trial Registration Data Set (Supplemental 14)
Protocol version	3	Date and version identifier (*pg4. line 42*)
Funding	4	Sources and types of financial, material, and other support (*pg14. line 43*)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (*pg3. line 9*)
	5b	Name and contact information for the trial sponsor (*pg3. line 54*)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities. (*pg14. line 33*)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (*pg4. line 60*)
	6b	Explanation for choice of comparators (*pg5. line 20-43*)
Objectives	7	Specific objectives or hypotheses (*pg8. line 14*)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (*pg4. line 26*)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (*pg6. line 59*)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (*pg7. line 7*)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (*pg7. line 17 and pg9. line 52*)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) (*pg10. line 43*)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (*pg7. line 52*)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Not available)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (*pg8. line 19*)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Table 3)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (*pg11. line 41*)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (*pg7. line 52*)
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions (*pg7. line 41*)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned (*pg7. line 45*)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (*pg7. line 52*)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Not applicable)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (Not applicable)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol (*pg11. line 20*)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols (*pg10. line 40*)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol (*pg11. line 22*)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Supplementary 15)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Supplementary 15)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) (Supplementay15)

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed (Supplementary 16)
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial (Supplementary 15)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct (Supplementary 15)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (Supplementary 16)

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (*pg12. line 35*)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) (*pg12. line 45*)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial (*pg12. line 48*)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (*pg14. line 51*)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators (Supplementary 16)
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions (*pg4. line 44*)
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates ("Informed Consent Form for RCT" and "Informed Consent Form for Tumor Tissue Biopsy")
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable (Supplementary 17)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Author notes: the above pages and line numbers refer to the first submission synthesized PDF, titled *bmjopen-2023-078049_Proof_hi*.